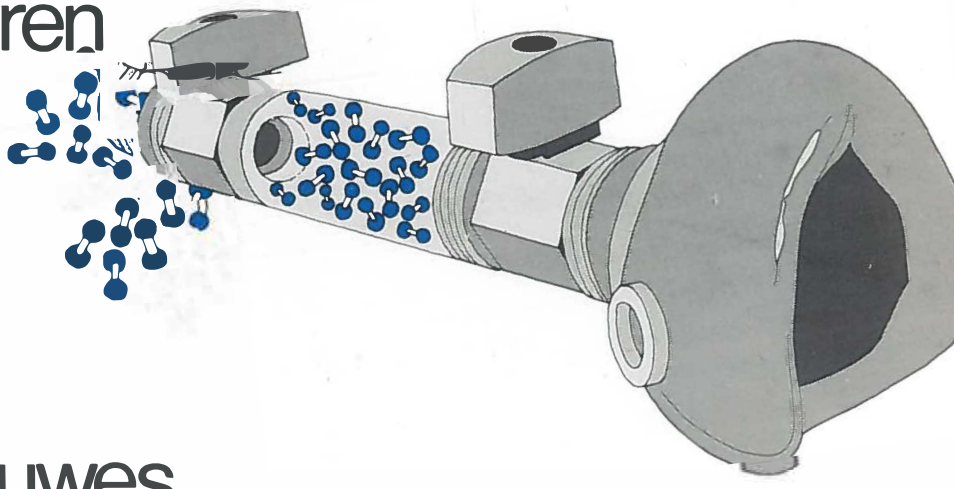
respiratory hydrogen excretion as a parameter for sugar malabsorption in children



a.c.douwes

RESPIRATORY HYDROGEN EXCRETION AS A PARAMETER FOR SUGAR MALABSORPTION IN CHILDREN

PROEFSCHRIFT

TER VERKRIJGING VAN HET DOCTORAAT IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE GRONINGEN OP GEZAG VAN DE RECTOR MAGNIFICUS DR. J. BORGMAN IN HET OPENBAAR TE VERDEDIGEN OP WOENSDAG 24 OKTOBER 1979 DES NAMIDDAGS TE 2.45 UUR PRECIES

DOOR

ADRIANUS CORNELIS DOUWES

GEBOREN TE UTRECHT

1979

DRUKKERIJ J.H. PASMANS B.V., 's-GRAVENHAGE

STELLINGEN

1. Bepaling van waterstof in uitademingslucht behoort een vaste plaats te hebben bij het onderzoek naar koolhydraat-malabsorptie in de kindergeneeskunde.

2. De combinatie: lactose-saccharose-arme voeding kan worden vervangen door lactose-arme voeding.

3. Het nut van vermindering van het lactosegehalte in zuigelingenvoeding is op zijn minst twijfelachtig.

4. Men moet onderscheid maken tussen suikermalabsorptie en suikerintolerantie, ook al is de overgang vloeiend.

5. Proefbehandeling met glutenvrije voeding zonder voorafgaande biopsie van de dunne darm, kan worden beschouwd als mishandeling (Lloyd-Still, J. Ped. Res. 95: 10, 1979).

6. Fiberoptische coloscopie bij kinderen behoeft geen anaesthesie en verschaft meer informatie dan het onderzoek met de stijve rectoscoop.

7. Kinderen met chronische colitis behoren te worden (mede-) behandeld door een gastroënterologisch geschoolde kinderarts.

8. De diagnose van psychogeen braken bij zuigelingen wordt eerder ingegeven door de arts-moeder relatie dan door de moeder-kind relatie.

9. De gangbare opvatting dat volledige intraveneuze voeding kort na grote operaties ten gevolge van stress-metabolisme niet mogelijk is, is onjuist en leidt tot onnodig gewichtsverlies.

10. Na "correctie" van een tetralogie van Fallot is evaluatie van het hemodynamisch resultaat niet mogelijk zonder het verrichten van een hartcatheterisatie (Garson et al., Circulation 59: 1232, 1979).

11. Melk moet, maar niet voor iedereen.

Groningen, 24 oktober 1979 A.C. Douwes

PROMOTOR: Prof.Dr. J. Fernandes
COPROMOTOR: Prof.Dr. T.D. Stahlie (VU - Amsterdam)
COREFERENT: Dr. J.H.M. van Tongeren (KU - Nijmegen)

Ontwerp omslag: Leo Creemers

aan Dineke
aan onze ouders

CONTENTS

Chapter 1

GENERAL INTRODUCTION

The history of the hydrogen breath test is a short one. Measurement of hydrogen in expired air has only recently been developed as a readily available and sensitive index of carbohydrate malabsorption.

The key publication of Levitt, showing the validity of the hydrogen breath test as a diagnostic procedure for the detection of lactose malabsorption, has been preceded by numerous papers reflecting an increased interest in the composition of human gaseous excreta, i.e. flatus and expired air, especially in relation to the consumption of wind producing foods like beans and onions.

This increase in research in a more or less neglected – at least by scientists – field of human digestive physiology, started in the sixties and was stimulated by the combined interest of NASA space flight medical engineers and by manufacturers of bean based food products. The results of these studies have been presented during a series of annual Dry Bean Conferences, from 1956 till 1968 (Annals New York Academy of Sciences, 1968, 150).

One of the many medical spin-off products of the American space flight programs is the now widely used synthetic, elemental diet (Vivonex) which provides adequate amounts of calories, minerals and vitamins with minimal production of faeces and flatus. Ironically, Vivonex has not been used in the Gemini- and Apollo flights because of its poor palatability (Penn, 1973).

Though Nielsen in 1961 described a gas chromatographical method for the detection of expired hydrogen and methane after consumption of beans, reproduced in 1966 by Doris Calloway, it was not until 1968 that Levitt using this method showed that not only beans, but also lactose instilled in the colon, gave rise to increased concentrations of hydrogen in expired air. This finding was not unexpected since Blackwood in 1956 and Calloway in 1966 demonstrated that large amounts of hydrogen (about 100 ml from 1 gram glucose) can be produced in vitro by bacterial fermentation of oligo- and monosaccharides. Calloway in 1969 was the first to report about the clinical relevance of measuring hydrogen excretion in mixed expired air for the detection of lactose intolerance.

The above cited publication of Levitt in the same year, subsequent to his intubation studies, was more fundamental and therefore more convin-

cing however. It made the rebreathing method the procedure of choice for subsequent investigators and a reference for the modified and simpler methods such as the end-expiratory air method (Metz, 1975, 1976) and the mixed expiratory air method for infants and children (Douwes, 1978).

In our country, the new diagnostic procedure has been introduced by Dolmans in 1978, using the rebreathing as well as the end-expiratory method for the detection of bacterial overgrowth of the small intestine.

The application of the hydrogen breath test in paediatrics started in 1975, in the Sophia Children's Hospital, Rotterdam (Head: Prof. Dr. H.K.A. Visser) with the use of a rebreathing system adapted to children (Vos, 1976). Maffei, who at this time collaborated with Metz, described in the same year the use of an adapted end-expiratory technique which, in contrast to the rebreathing method, could also be used in infants and very young children.

Since we were aware of the limitations of the rebreathing technique in paediatric patients which requires their active cooperation, we developed an alternative method, using mixed expired air samples. This non invasive method is suitable for infants and children of all ages. Our experience with the rebreathing method, the development of the mixed expired air method and its multipurpose applications are the components of this thesis.

About the origin of expired hydrogen

Hydrogen in expired air (H_2) originates mainly from the fermentation of non absorbed carbohydrates by the resident intestinal microflora (Fig. 1).

Since human metabolism does not produce H_2 and atmospheric air contains only 0.5 parts per million H_2 (0.5 ml per 1000 L), this molecule is ideally suited to serve as a non isotope index of carbohydrate malabsorption. Specificity and sensitivity are similar to the $^{14}CO_2$-breath test (20).

Correlation between intestinal H_2 production and pulmonary excretion is fairly constant ($r = 0.94$) and it is generally assumed that 14% of the total H_2 production is excreted by the lungs (13, 14). This however, probably is an underestimation since in Levitt's study large quantities of H_2 were washed out by constant perfusion of the bowel with argon, via a tube with its tip located at the Treitz ligament. Under normal conditions probably 21% of the intestinal H_2 production appears in the expired air (14).

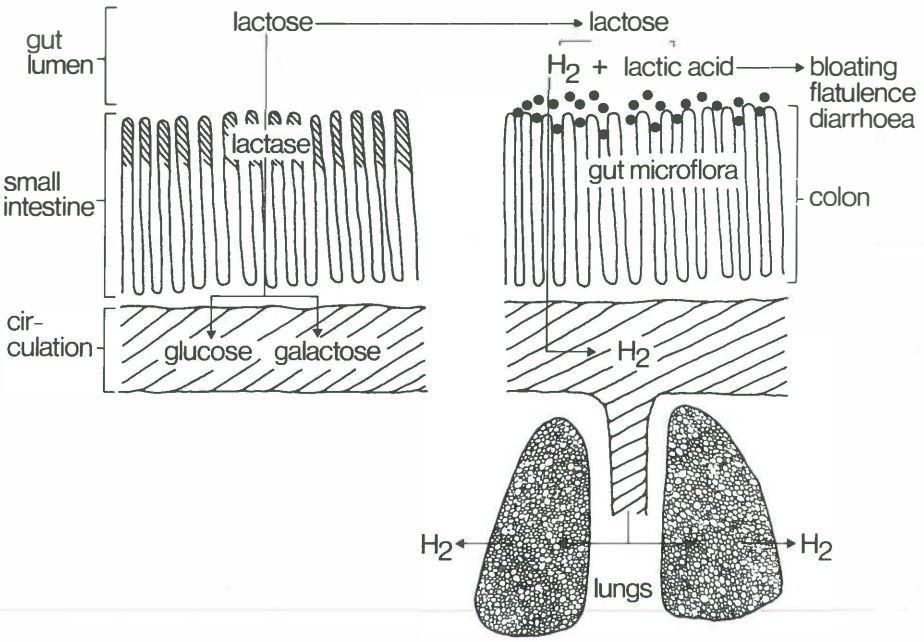


Fig. 1. Schematic drawing of normal lactose digestion and absorption and of lactose malabsorption with bacterial fermentation and generation of H_2.

In the absence of substrate, generation of H_2 by gut bacteria is very low in vivo and in vitro and supplying mono- or oligosaccharides to human intestinal flora results in the generation of large amounts of H_2 and CO_2 in vitro (2, 5) as well as in vivo (13). The chemical events leading to this production have been described in detail (17).

The volume of H_2 generated depends primarily on the amount of fermentable substrate in the colon (14), thus this amount can be calculated from the expired H_2 (3). Hence, the H_2 breath test provides semi-quantitative information about the severity of carbohydrate malabsorption and can also be used to estimate reciprocally the dose of carbohydrate that can be absorbed by the individual malabsorber.

Many bacterial strains are capable to ferment sugars and to generate H_2 as one of the metabolic end products. The predominating faecal flora mainly consists of non-sporeforming anaerobes; these are present in billions per gram and outnumber the aerobes by at least 10^3 (10). It is therefore not surprising that antibiotic suppression of this microflora results in the decrease of disappearance of the H_2 response to the non

absorbable sugar lactulose (12). Since so may bacteria are H_2 producers, it is difficult to understand why about 2% of the persons who are not receiving antibiotics likewise do not excrete H_2 after ingestion of lactulose (4, 15). In our patient material of over 200 children, one was found incapable to ferment lactulose (Duphalac) which therefore could not act as a laxative. The absence of H_2 excretion after lactulose in a small proportion of the population may in part be due to the interference of hydrogen bacteria which use H_2 as oxidizable substrate (Pseudomonas) and methane bacteria, producing CH_4 from H_2 and CO_2 (24, 27). The activity of H_2 utilizing bacteria may also explain the differences in H_2 response between individual malabsorbers, but this awaits further elucidation. Reproducibility of the H_2 response in the individual malabsorber at subsequent occasions is fairly good (11, this study chapter 2, Fig. 2).

Though unimportant for tolerance tests with selected sugars, the question whether expired H_2 exclusively originates from bacterial fermentation of sugars or also from proteins and fats, is relevant when whole foods are used to study carbohydrate malabsorption (chapter 7). Information about H_2 production from fats is not available from literature. Incubation of 1-2 ml of corn oil with 3-4 grams of freshly passed faeces suspended in 5 ml 0.9% NaCl, kept at 37% for 24 hours in rubber sealed 14 ml capacity glass tubes did not give rise to a detectable production of H_2, whereas 1 granule of white sugar sufficed to generate abundant amounts of H_2 (Douwes, 1979, unpublished observations).

The gaseous end products of bacterial aminoacid metabolism are mainly NH_3, CH_4 and CO_2 (5). Yet, the possibility of some H_2 production from aminoacids cannot be excluded. Clostridia, for example, can produce H_2 via the Stickland reaction, a coupled oxidation-reduction between pairs of aminoacids (27). Incubation of 1 gram of arginine with colonic dejecta under anaerobic conditions, generated 7 ml of gas, containing 9% (0.6 ml) H_2 (5). On the other hand, we failed to demonstrate any H_2 production in the test tubes after incubation of faeces with 1 gram of carbohydrate-free soy protein hydrolysate (CHO-FREE). Hence, the conclusion seems warranted that expired H_2 almost exclusively originates from bacterial fermentation of non absorbed carbohydrates.

Methods of sampling and storing expired air and estimation of H_2

The concentration of H_2 can be measured gaschromatographically, using a thermal conductivity detector (TCD) in *rebreathed expired* air

(14, 20), in *end-expiratory air* (19) or in *mixed expired air* (9, 22, 25).

Expired air samples can be analysed directly or stored in 4-6 L capacity, multilaminar gastight bags (6, 11, 26), in rubber sealed vacuum glass tubes (Vacutainer) (9, 20) or for one or two days, in a plastic syringe (8).

Most collecting systems require the active cooperation of the patient who has to fill a gastight bag with expired air, to rebreath in a closed system for 3-5 minutes through his mouth with his nose clamped, or to exhale by mouth into a Haldane-Priestly tube. None of these procedures can be used in infants and children under the age of four years.

The development of the end-expiratory air method (19) enabled an adaption to this age group by collecting end-expiratory air via a nasal tube with the tip located in the oropharynx (16) or with a nasal prong (1). The first fully non invasive procedure, measuring H_2 concentrations in mixed expired air, makes use of a paediatric size face mask attached to a 5 L capacity gas bag (25, 26) or a paediatric face mask with lateral inspiration porthole attached to an open ended, 28 ml capacity plastic collection pipe (9).

This delayed development of appropriate paediatric sampling procedures is largely due to the technical problem of accurately measuring trace amounts of H_2 present in end or mixed expiratory air samples because the TCD was generally believed too insensitive to quantitate H_2 at the low concentrations involved. Only the use of the helium ionization detector (HID) could overcome this problem (7, 11). Since the HID is more expensive than the TCD, presents some radiation hazard and its maintenance is difficult, most investigators have resorted to the rebreathing procedure which enables to concentrate H_2 to the extent that it can be by measured by TCD. Publications describing gaschromatrographical procedures with use of the TCD to detect H_2 concentrations in the 10 ppm range to an accuracy of 2 ppm, date from the last 3 years (8, 9, 19). This reintroduction of the TCD as a sensitive detector for H_2 measurements provides the basis for clinical application of the H_2 breath test in paediatrics.

References

1. Barr, R.G., Perman, J.A., Schoeller, D.A. and Watkins, B. Breath tests in pediatric gastrointestinal disorders: new diagnostic opportunities. Pediatrics, 62, 393 (1978).
2. Blackwood, A.C., Neish, A.C. and Ledingham, G.A. Dissimilation of glucose at controlled pH values by pigmented and non pigmented strains of Escherichia coli. Journal of Bacteriology, 72, 497 (1956).

3. Bond, J.H., Levitt, M.D. Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate malabsorption. Journal of Clinical Investigation, 51, 1219 (1972).
4. Bond, J.H., Levitt, M.D. Investigation of small bowel transit time in man utilizing pulmonary hydrogen (H_2) measurements. Journal of Laboratory and Clinical Medicine, 85, 546 (1975).
5. Calloway, D.H., Colasito, D.J., Mathews, R.D. Gases produced by human intestinal microflora. Nature, 212, 1238 (1966).
6. Calloway, D.H., Murphy, E.L. The use of expired air to measure intestinal gas formation. Annals New York Academy of Sciences, 150, 82 (1968).
7. Calloway, D.H., Murphy, E.L., Bauer, D. Determination of lactose intolerance by breath analysis. American Journal of Digestive Diseases, New Series, 14, 811 (1969).
8. Dolmans, W. H_2-excretie in de uitademingslucht bij koolhydraatmalabsorptie. Thesis, Nijmegen (1978).
9. Douwes, A.C., Fernandes, J. and Rietveld, W. Hydrogen breath test in infants and children: sampling and storing expired air. Clinica Chimica Acta, 82, 293 (1978).
10. Gall, L.S. Study of the normal faecal bacterial flora of man. NAS_W-738. NASA. Washington, D.C. cited from: Gall, L.S. The role of intestinal flora in gas formation. Annals New York Academy of Sciences, 150, 27 (1968).
11. Gearhart, H.L., Bose, D.P., Smith, C.A. and Morrison, R.D. Determination of lactose malabsorption by breath analysis with gas chromatography. Analytical Chemistry, 48, 393 (1976).
12. Gilat, T., Ben Hur, H., Gelman-Malachi, E., Terdiman, R. and Peled, Y. Alterations of the colonic flora and their effect on the hydrogen breath test. Gut, 19, 602 (1978).
13. Levitt, M.D. and Ingelfinger, F.J. Hydrogen and methane production in man. Annals New York Academy of Sciences, 150, 75 (1968).
14. Levitt, M.D. Production and excretion of hydrogen gas in man. The New England Journal of Medicine, 281, 122 (1969).
15. Levitt, M.D., Donaldson, R.M. Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. Journal of Laboratory and Clinical Medicine, 75, 937 (1970).
16. Maffei, H.V.L., Metz, G.L., Jenkins, D.J.A. Hydrogen breath test: adaptation of a simple technique to infants and children. Lancet i, 1110 (1976).
17. Mayhew, S.G., Veeger, C. Enzymatische vorming en oxidatie van H_2. Chemisch Weekblad, juli, 377 (1976).
18. Metz, G., Jenkins, D.J.A. Peters, T.J., Newman, A., Blendis, L.M. Breath hydrogen as a diagnostic method for hypolactasia. Lancet, i, 1155 (1975).
19. Metz, G., Gassull, M.A., Leeds, A.R., Blendis, L.M. and Jenkins, D.J.A. A simple method of measuring breath hydrogen in carbohydrate malabsorption by end-expiratory sampling. Clinical Science and Molecular Medicine, 50, 237 (1976).
20. Newcomer, A.D., McGill, D.B., Thomas, P.J., Hofmann, A.F. Prospective comparison of indirect methods for detecting lactase deficiency. New England Journal of Medicine, 293, 1232 (1975).
21. Nielsen, J.P. Trace constituents in breath as related to flatulence. Proceedings of the Fifth Annual Dry Bean Research Conference convened by the Western Regional Research Laboratory, U.S. Dept. of Agriculture, Denver, Colorado, 49 (1961).
22. Payne-Bose, D., Tsegaye, A., Morrison, R.D. and Waller, G.R. An improved method for determining breath H_2 as an indicator of carbohydrate malabsorption. Analytical Biochemistry, 88, 569 (1978).
23. Penn, H.J. Ruimtevaart en Geneeskunde, Philips-Duphar Nederland B.V., Amsterdam, 77 (1973).
24. Schlegel, H.G. Algemeine Mikrobiologie. Thieme, Stuttgart, 225 (1972).
25. Solomons, N.W., Viteri, F.E. and Hamilton, L.H. Application of a simple gas chromatographic technique for measuring breath hydrogen. Journal of Laboratory and Clinical Medicine, 90, 856 (1977).
26. Solomons, N.W. and Viteri, F., Rosenberg, I.H. Development of an interval sampling hydrogen (H_2) breath test for carbohydrate malabsorption in children: evidence for a circadian pattern of breath H_2 concentration. Pediatric Research, 12, 816 (1978).
27. Stanier, R.Y., Doudoroff, M., Adelberg, E.A. General Microbiology. Prentice-Hall, Inc., Englewood Cliffs, New Yersey, U.S.A, 192 and 644 (1970).
28. Vos, C.E. Proceedings annual meeting of the European Society for Paediatric Gastroenterology and Nutrition (1976).

Chapter 2

RESPIRATORY HYDROGEN EXCRETION AS A PARAMETER FOR LACTOSE MALABSORPTION IN CHILDREN

J. FERNANDES, C.E. VOS, A.C. DOUWES, E. SLOTEMA and H.J. DEGENHART

Abstract

Respiratory hydrogen excretion was measured during tolerance tests with lactose, glucose plus galactose, and skim milk in 52 children, 4 to 15 years of age. Ten children appeared to be lactose-malabsorbers, as reflected by increased respiratory hydrogen excretion after administration of 2 g lactose per kilogram, maximum 50 g. Skim milk, equivalent to 0.5 g lactose per kilogram was administered to all lactose-malabsorbers. Eight children were tolerant and two children were "intolerant" for this physiological amount of lactose when administered as skim milk. Disaccharidase activities of jejunal biopsies were determined in all 10 children with lactose malabsorption. Lactase activity was deficient in nine children and normal in one child. The increase of blood glucose during the lactose tolerance test did reflect lactose malabsorption less accurately than the respiratory hydrogen excretion.

Of various methods to detect lactose malabsorption in adults the measurement of respiratory hydrogen excretion (RHE) has been shown to be the most sensitive (1, 2). This observation is based on the fact that mainly H_2 is formed from nonabsorbed carbohydrate residue in the colon (3) and that approximately 14% of intestinal H_2 diffuses to the blood and thence to the expired air (4). Even the amount of lactose that can be tolerated without side effects can be estimated by measuring RHE after ingestion of various amounts of lactose (5).

The measurement of RHE seemed to us very suitable for application in children for several reasons. Malabsorption of lactose in children may give rise to vague abdominal symptoms. The symptoms, however, depend on the degree of fermentation of non-absorbed lactose as indicated by the RHE rather than on the degree of absorption as indicated by changes

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in the blood glucose. Further , the measurement of RHE might be used to titrate the child's tolerance for different amounts of lactose. Therefore we compared RHE during the lactose tolerance test (2 g lactose per kilogram body weight) and after skim milk administration (0.5 g lactose per kilogram body weight). This might clarify if children, intolerant for large amounts of lactose, might be tolerant for physiological amounts of milk. The determination of RHE is a noninvasive method and therefore especially suitable to be used in children.

Patients and methods

RHE was measured in children suspected of having lactose maldigestion or malabsorption. Fifty-six tests were performed on 52 children, 4 to 15 years of age. Patients younger than 4 years could not be investigated, as cooperation of the child for 3 min breathing in a rebreathing system was necessary.

The symptons and diagnoses of the patients were recurrent diarrhea of unknown cause, abdominal pain, giardiasis, celiac disease, cystic fibrosis, Crohn's disease, and short bowel syndrome.

Lactose (2 g/kg body weight, maximum 50 g), the component monosaccharides (each 1 g/kg), or skim milk (equivalent to 0.5 g lactose per kilogram) were administered after an overnight fast.

Our normal values for blood glucose are an increase $>$ 1.2 mM after lactose administration (2,5).

RHE was measured using a rebreathing technique to concentrate hydrogen in the expired air. Every 30 min the patient breathed into a closed system for a period of 3 min. This procedure took place over 2.5 hr (six determinations). The total volume of the closed breathing system comprised a balloon inflated to 600 to 1200 ml, tubing and connecting pieces including a CO_2 absorber amounting to 0,5 liter and the functional residual capacity (FRC) of the patient as estimated from a nomogram (6, 7). Oxygen was supplied during the 3 min in an amount equivalent to the consumption (derived from the nomogram). H_2 was considered to be evenly distributed throughout the system. The total system volume multiplied by the concentration of H_2 in the balloon, was defined as volume of H_2 excreted over the 3 min period.

The formula used was:

$$H_2 \text{ production during 3 min} = (FRC + A + B) \times H_2 \text{ concentration}$$

FRC = functional residual capacity
A = tubing, connective pieces, CO_2 absorber
B = balloon volume

The error in the FRC value can be ± 30% (2 SD). This is a systematic error. The error in "A" is ± 1% (2 SD) and is also systematic. The errors in "B" and in the H_2 concentration measurement are random errors, approximately 7 and 5% (2 SD), respectively. The resulting random error in the H_2 production measurement can be calculated according to standard techniques (8). The magnitude of this error varies between 5 and 10%.

Another source of error might be due to preferential leakage of H_2 through the rubber balloon. Therefore we filled the rubber balloon with 195 ppm H_2: the recovery appeared to be complete after 30 min. As we injected all air samples into the gas chromatograph within 3 min, leakage of H_2 through the rubber, could not have been of any significance.

The H_2 concentration in the samples of expired air was determined by gas chromatography (Varian Aerograph model 90-P3) using a thermal conductivity detector. A 5 ml sample of gas was taken from the collecting system and injected through a gas sampling valve into a stainless steel column. 5 feet by ¼ inch, packed with molecular sieve 5 Å . The detector and injector temperature was 100 C. Column temperature was 30 C. Argon, 25 ml/min, served as the carrier gas. The pressure was 3 atm. Reference gas mixtures (Hoek Loos, Amsterdam, the Netherlands) contained 195, 860, and 1760 ppm H_2 in N_2. The reference gas mixtures were certified for purity (> 99.9%). As regards the accuracy, the concentration was certified not to differ more than 1% from the concentration that was ordered.

Our normal values for RHE after lactose loading are < 0.1 ml/min. (Fig. 1) (1).

Capillary blood was taken for glucose determination in the fasting state and after lactose ingestion (not after skim milk administration) at ¼, ½, 1, 1½, 2, and 2½ hr. A standard glucose oxidase method was used.

Jejunal biopsy was performed in all children with RHE > 0.1 ml/min after lactose and in some children in whom the interpretation of the normal results was in doubt. Disaccharidase activities of jejunal biopsies were determined according to Dahlquist (9).

Informed consent was obtained from the parents for both the determination of RHE and jejunal biopsy. Parents were present during the procedures if they so desired. The procedures were also carefully explained to the children.

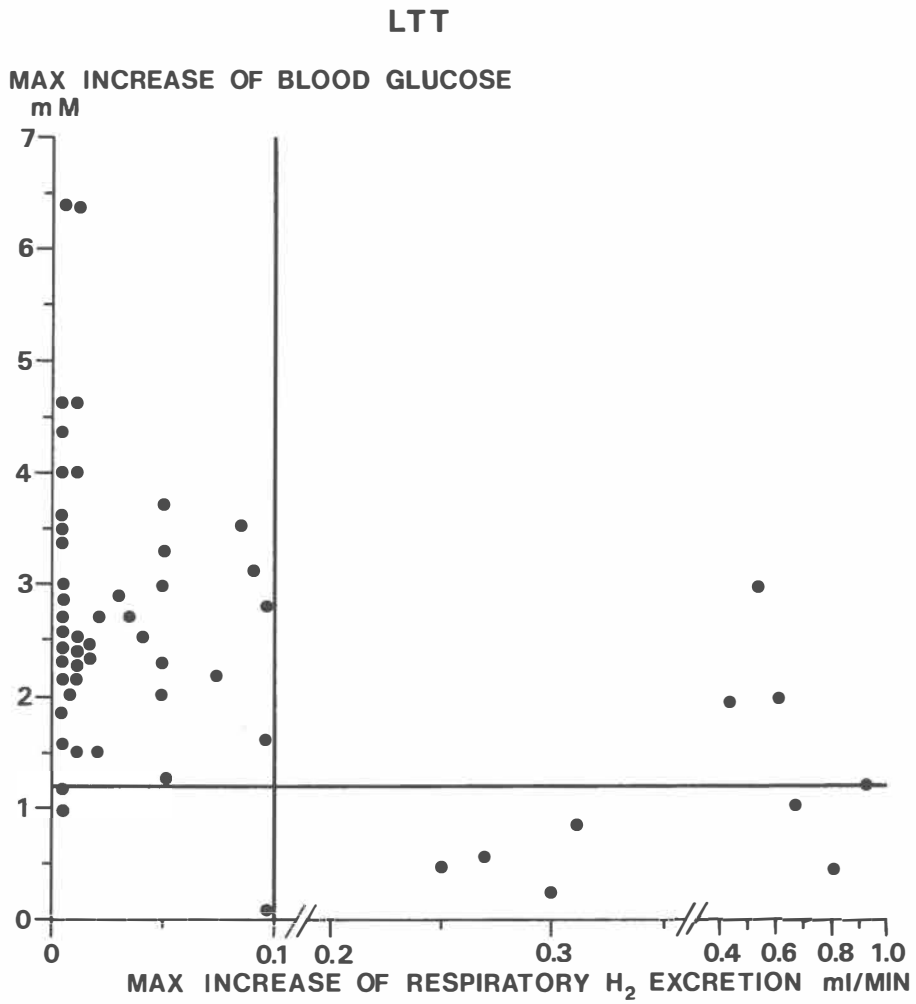

Fig. 1. Lactose test performed in 52 patients. Each point represents the maximal increase of respiratory H_2 excretion and the maximal increase of blood glucose per child after lactose administration (2 g/kg, maximum 50 g).

Results

Fifty-six lactose tolerance tests were performed in 52 patients. The results are shown in Figure 1 in a manner similar to that of Levitt and Donaldson (5). The 42 patients with a low RHE (< 0.1 ml/min) showed

a normal increase of blood glucose (> 1.2 mM), with the exception of three children. The interval between the patients with normal and abnormal RHE was at least 0.15 ml/min, an easily detectable difference.

The disaccharidase activities of the jejunal biopsies of all 10 patients with increased RHE are shown in Table 1. All subjects were lactase-deficient with the exception of patient 1. Disaccharidase activities of the children with normal RHE (< 0.1 ml/min) and normal increase of blood glucose (> 1.2 mM) after lactose loading were not measured except in three children, who showed normal lactase activities. Disaccharidase activities of the three children with subnormal or borderline increase of blood glucose (≤ 1.2 mM) and normal RHE (< 0.1 ml/min) were not measured. On follow-up, these children showed no abnormalities with a normal milk content of the diet.

The RHE and blood glucose curves of patient 3 after lactose and after glucose plus galactose administration are shown in Figure 2. This patient with frequent abdominal pain was accustomed to drinking milk in order to relieve her pain as did her father for his gastric ulcer. It appeared that her RHE increased abnormally during two lactose tests, performed with an interval of 2 months. The blood glucose curves were normal on both occasions. The H_2 overproduction did not occur after administration of glucose plus galactose, nor after sucrose (data not given).

Table I: Jejunal disaccharidase activities of 10 children with lactose malabsorption[a]

Patient	Age	Diagnosis	Lactase	Sucrase	Isomaltase	Maltase	Protein
	yr						
1	13	Crohn's disease	1.3	6.7	12.0	22.3	92
2	5	Celiac disease	0.0	0.9	1.6	4.1	12
3	11	Late onset lactase deficiency	0.4	5.5	7.9	22.5	71
4	4	Secondary lactase deficiency	0.4	3.9	4.8	15.9	85
5	6	Diabetes mellitus villous atrophy	0.0	0.4	0.5	3.3	24
6	4	Secondary lactase deficiency	0.7	8.8	9.4	30.6	64
7	12	Secondary lactase deficiency	0.0	7.6	9.3	25.6	62
8	13	Secondary lactase deficiency	0.2	3.9	3.5	24.4	36
9	11	Secondary lactase deficiency	0.6	8.5	8.1	30.2	54
10	15	Chrohn's disease	0.3	4.9	2.5	16.1	72
Normal values			0.9-7.3	1.9-10.3	2.1-8.0	7.5-35.2	

[a] The enzyme activities (μmoles disaccharide split per minute per gram of mucosa) were determined according to Dahlquist (9). Normal values were taken from Townley et al. (10), who used the same technique. Protein content was determined according to Lowry et al. (11) and expressed in mg protein per gram of mucosa (wet weight).

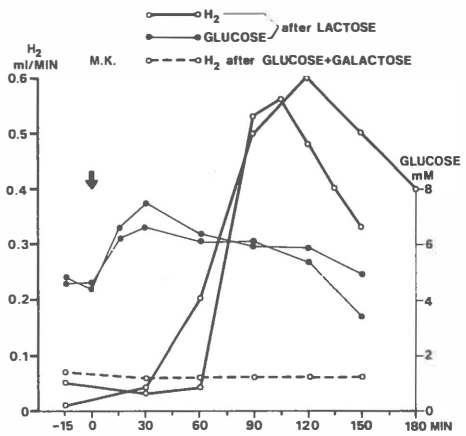

Fig. 2. Lactose and glucose plus galactose tests in patient 3 with late onset lactase deficiency.

The RHE curves of patient 9 after lactose and after skim milk administration are shown in Figure 3. The patient had secondary lactase deficiency. Although the patient had abdominal pain and markedly increased RHE during the lactose test, he showed neither increase of RHE nor untoward symptoms after skim milk ingestion. The RHE curves of patient 10 after lactose and after skim milk administration are shown in Figure 4. This patient with Crohn's disease had no diarrhea at the time of investigation. Increased RHE associated with nausea followed lactose administration (the data of the flat glucose curve are not shown). The administration of skim milk was also followed by increased RHE, without side effects, however.

Discussion

In order to diagnose lactase deficiency, many authors rely on the relationship between the increase of blood glucose after lactose loading on the one hand and lactase activity of the jejunum on the other hand. However, others (1, 12-14) stress the discrepancies between these parameters. The enzyme activity, measured in the jejunal biopsy, need not necessarily reflect total lactase activity of the jejunum, which may be influenced by the length of the small intestine, impairment of ileal function (ileitis), patchy lesions of the jejunum.

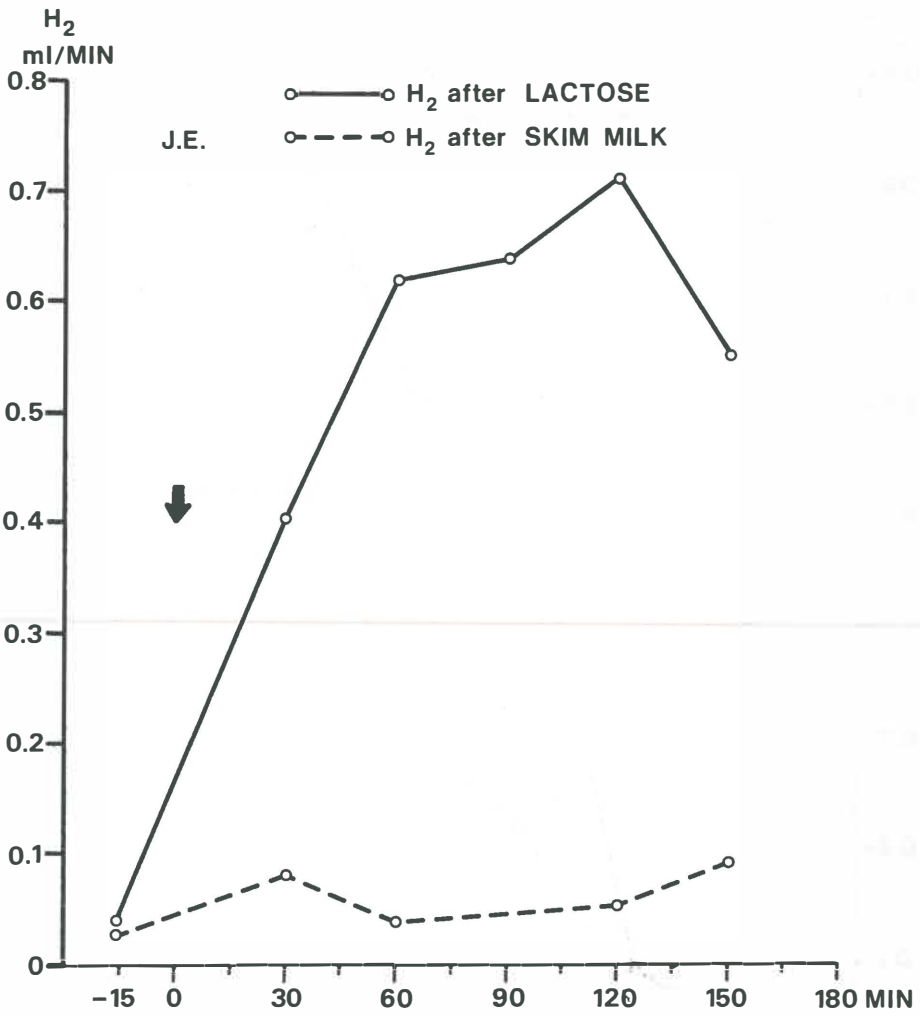

Fig. 3. Lactose and skim milk tests in patient 9 with secondary lactase deficiency.

It can further be deduced from the literature that much confusion exists about the terminology of lactose malabsorption and lactose intolerance. The term lactose malabsorption should be used when lactose ingestion is followed by a flat glucose curve. The term lactose intolerance should be used when gastrointestinal symptoms occur during or after the test. Lactose intolerance may occur despite a normal glucose peak (> 1.2 mM) and lactose tolerance despite a flat glucose curve (12-14).

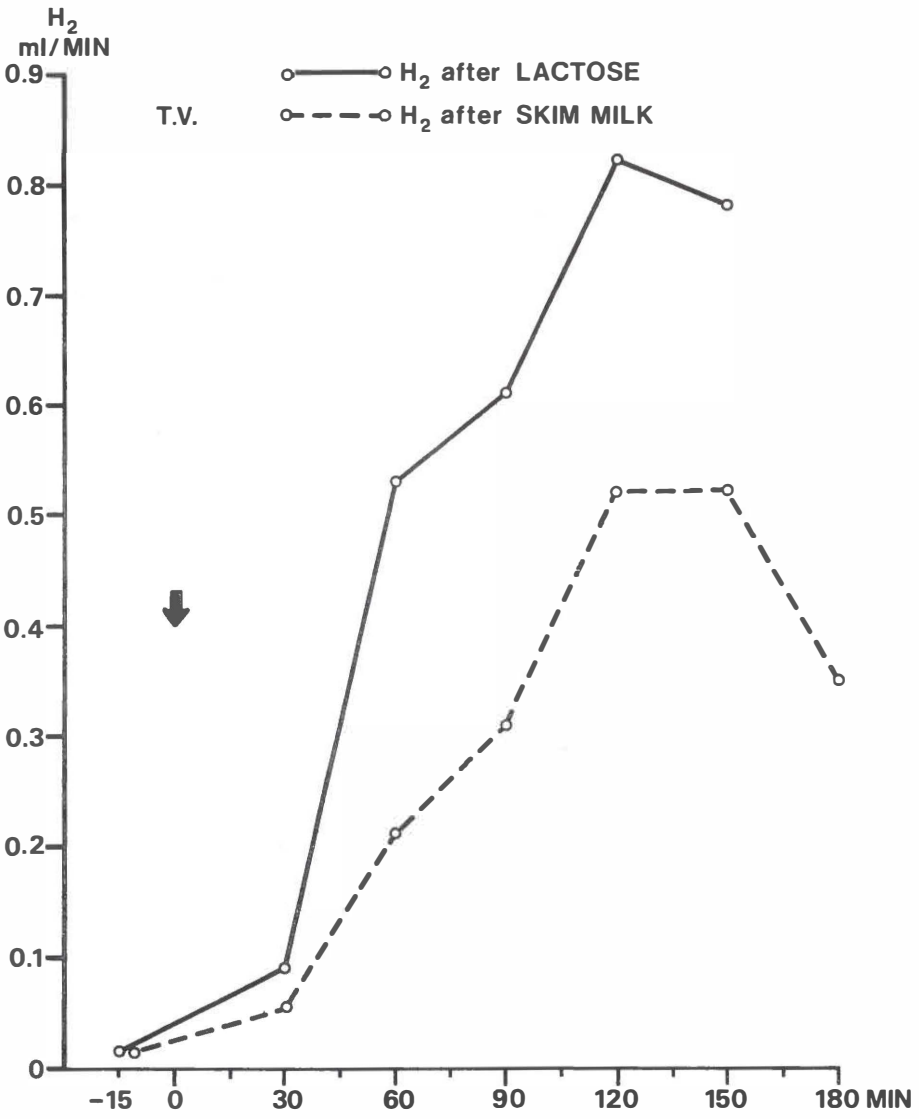

Fig. 4. Lactose and skim milk tests in patient 10 with Crohn's disease.

Harrison and Walker-Smith (14) even stated that "lactose intolerance (in children) occurred whether the rise in blood glucose was flat, borderline or normal – that is, there was no correlation between maximum rise in blood glucose and clinical lactose intolerance, nor indeed was there

any constant relationship between this rise and the presence of reduced lactase activity or the state of the small intestinal mucosa".

As we did not intend to evaluate the opposite conclusions of different authors, we took a different approach to the problem. Most tolerance tests are performed with the aim of assessing the quantity of lactose absorbed. It would be more relevant, however, to submit the patient to a tolerance test, which reflects the quantity of lactose that is not absorbed, the malabsorbed sugar being the cause of the patient's complaints. In this respect increased RHE after lactose ingestion seems to be a sensitive and accurate indicator for lactose malabsorption (1, 2, 5). In addition the test is more specific for lactose intolerance because the large quantities of H_2 formed by fermentation are probably a factor in gaseous distention of the bowel (4).

Taking these considerations into account, the aim of our investigations was two-fold. First, to study the relationship between lactose malabsorption (increased RHE), lactose intolerance (intestinal symptoms), peak blood glucose after lactose ingestion, and jejunal lactase activity. Second, to differentiate the effects of different lactose loads by comparing RHE after ingestion of 2 g lactose per kilogram (maximum 50 g) in water and after 0.5 g lactose per kilogram, administered as skim milk. This might have practical implications for the child's diet. In the present study increased RHE after ingestion of 2 g lactose per kilogram was found in 10 patients. In nine of these abdominal symptoms occurred. One patient had no complaints or symptoms of discomfort. Thus 10 patients were lactose malabsorbers and the majority were lactose-intolerant as well. Of the 10 children the nine with symptoms were lactase-deficient while the one without symptoms had normal lactase activity (Table 1). Of the 10 children with increased RHE after lactose ingestion three had normal peak blood glucose > 1.2 mM. Two of these were lactose intolerant, the third was the patient with lactose malabsorption and normal lactase activity. This patient with Crohn's disease might have lactose malabsorption (increased RHE) due to insufficient total lactase activity of the small bowel (ileitis), or due to bacterial overgrowth, and either possibility is compatible with a normal lactase activity of a proximal jejunal biopsy. Similar discrepancies between lactase and peak blood glucose after lactose ingestion were noted in adults (1). Realizing that the transition of lactose malabsorption into normal absorption could be dose dependent (5, 15, 16), we determined RHE after administration of skim milk (0.5 g lactose per kilogram) to the 10 lactose malabsorbers. This time none showed any side effects ("intolerance"). RHE

was still markedly increased in two patients, one of whom is shown in Figure 4. In the other eight patients RHE was normal. Sensu strictiori the two milk-malabsorbers were not milk-intolerant. This would mean that physiological amounts of milk should be permitted to all our patients, irrespective of the fact that they were lactose intolerant and lactase deficient. This illustrates again, how difficult it is to differentiate between malabsorption and intolerance based on the clinical symptoms which are very difficult to evaluate during a single test.

We suspected that the two milk malabsorbers would be prone to hyperproduction of intestinal gas, abdominal distention, and other side effects, even if permitted physiological amounts of milk. Therefore, a milk-restricted diet was prescribed to the two children with increased RHE after skim milk, whereas the other eight patients, who could absorb skim milk without increased RHE, were allowed physiologal amounts of milk, spread over the day.

Though it is well-known that illness may impair carbohydrate digestion and absorption, such influences were carefully excluded in our patients, who were only studied in the "stable phase" of their illness. This is confirmed by the reproducibility of the tests, performed in several patients with considerable intervals (3 to 12 months) between each test.

Finally, it should be stressed that an exceptional patient with lactose malabsorption will have a false negative RHE after lactose ingestion (one out of 50 adults) (5). Thus, in case of doubt, a normal RHE after lactose may be coupled with a test to show that the patient can produce H_2. Determination of RHE after ingestion of lactulose, which is not absorbed (2, 3), or xylose, which is only partially absorbed (5) will eliminate false negative results.

While the complexity of H_2 determinations limits the scope of its use, the development of more sensitive instruments, such as the helium ionization detector and the measurement of H_2 in endexspiratory air may render the test suitable for children of all ages (17) and for sending small exspiratory air samples from field investigations to central laboratories.

Acknowledgements

The authors thank Messrs. R.J. Kraaipoel and T.C. Jansen for valuable technical help.

References

1. Newcomer, A.D., McGill, D.B., Thomas, P.J. and Hofmann, A.F. Prospective comparison of indirect methods for detecting lactase deficiency. New Engl. J. Med. 293: 1232, 1975.
2. Bond, J.H. and Levitt, M.D. Quantitative measurement of lactose absorption. Gastroenterology 70: 1058, 1976.
3. Levitt, M.D. and Bond, J.H. Volume, composition and source of intestinal gas. Gastroenterology 59: 921, 1970.
4. Levitt, M.D. and Ingelfinger, F.J. Hydrogen and methane production in man. Ann. N.Y. Acad. Sci. 150: 75, 1968.
5. Levitt, M.D. and Donaldson, R.M. Use of respiratory hydrogen (H_2) excretion to detect carbohydrate malabsorption. J.Lab. Clin. Med. 75: 937, 1970.
6. Doershuk, C.F., Downs, T.D., Matthews, L.R.W. and Lough, M.D. A method for ventilatory measurements in subjects 1 month-5 years of age: normal results and observations in disease. Pediat. Res. 4: 165, 1970.
7. Engström, I., Karlberg, P. and Swarts, C.L. Respiratory studies in children: Relationships between mechanical properties of the lungs, lung volumes and ventilatory capacity in healthy children 7-15 years of age. Acta Paediat. 51: 68, 1962.
8. Findlay, A. Practical Physical Chemistry, edited by J.A. Kitchener, 8th ed., New York: Longmans Green, 1954.
9. Dahlquist, A. Assay of intestinal disaccharidases. Enzym. Biol. Clin. 11: 52, 1970.
10. Townley, R.R.W., Khaw, K.T. and Shwachman, H. Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood. Pediatrics 36: 911, 1965.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265, 1951.
12. Krasilknikoff, P.A., Gudmand-Høyer, A.E. and Moltke, H.H. Diagnostic value of disaccharide tolerance tests in children, Acta Paediat Scand. 64: 693, 1975.
13. Garza, C. and Scrimshaw, N.S. Relationship of lactose intolerance to milk intolerance in young children. Am. J. Clin. Nutr. 29: 192, 1976.
14. Harrison, M. and Walker-Smith, J.A. Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity, and lactose tolerance tests. Gut 18: 48, 1977.
15. Calloway, D.H., Murphy, E.L. and Bauer, D. Determination of lactose intolerance by breath analysis. Am. J. Digest Diseases 14: 811, 1969.
16. Gearhart, H.L., Bose, D.P., Smith, C.A., Morrison, R.D., Welsh, J.D. and Smalley, T.K. Determination of lactose malabsorption by breath analysis with gas chromatography. Anal. Chem. 48: 393, 1976.
17. Maffei, H.V.L., Metz, G.L. and Jenkins, D.J.A. Hydrogen breath test: adaption of a simple technique to infants and children. Lancet i: 1110, 1976.

Chapter 3.A

DEVELOPMENT OF INTERVAL SAMPLING, MIXED EXPIRED BREATH H_2 TEST

Introduction

The clinical use of H_2 estimations in mixed expired air for the detection of lactose malabsorption has been described in 1969 by Calloway. She used Nielsen's procedure of gas chromatographical analysis with the high sensitivity helium ionization detector (HID), sampling expired air in 4 L capacity breath bags. This method has been reintroduced by Gearhart in 1976.

Theoretically, H_2 estimation in mixed expired air offers an opportunity for non invasive application in very young children and infants. However, the need for the HID and the large volumes of expired air necessary form serious practical limitations. Hence, the following problems had to be solved:

1. increase in sensitivity of the gas chromatographical assay using a thermal conductivity detector (TCD).
2. non invasive collection system with small volume and small dead space.
3. non-expensive storage procedure for small volumes of expired air.
4. assessment of the reliability of the new method.

Increase of sensitivity of gas chromatographical assay

The TCD or katharometer consists of a metal block drilled with separate channels through which the column effluent and pure carrier gas flow. Each channel carries an axial wire which is connected in opposite arms of a Wheatstone bridge circuit, fed from a constant current supply. When a peak passes one wire, the thermal conductivity differs from the other, giving rise to increased wire resistance. This is arranged to generate a positive peak on the recorder chart. The sensitivity of the TCD to a compound can be enhanced by:

a) increasing the difference in temperature between the wire and the block.

b) use of a carrier gas with large difference in thermal conductivity as compared to H_2. Argon or nitrogen are the preferable gases whereas the use of helium would not allow analysis of H_2 below 1% (Bulletin 760, Supelco, Inc.).

Base line drift however is the big problem with TCD's, especially when subjected to high temperatures. This problem could be solved by us by keeping conditions stable, also when the gas chromatograph is not in use. Only the wire temperature is reduced in order to prevent early breakdown of the TCD and a small flow of carrier gas prevents the room air to contaminate the column and the TCD.

Unfortunately, H_2 is eluted from the column so rapidly that the baseline upset, which occurs after injection of the sample, is difficult to distinguish from the H_2 peak. Rather dense packing of a rather long column with fine molecular sieve material (detailed in 3.B), low carrier gas-flow rate and low oven temperature largely overcomes this problem. Yet, not every column prepared in the same way provides the same results and separation of the H_2 peak from the signal caused by the injection pressure wave tends to become less with aging of the column. Fig. 1 shows the results of a well-prepared column after injection of reference gas-mixtures, containing different concentrations of H_2 in nitrogen. It takes 8-10 minutes before the gas chromatograph is ready for analysis of the next sample.

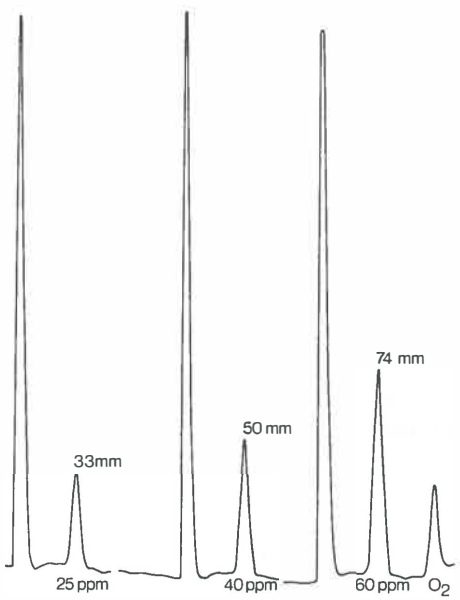

Fig. 1. Separation of hydrogen peak from injection peak, using three different reference gases and Varian Aerograph 90 P.

Reproducibility of H_2 determinations

This has been assessed by serial analysis of reference gases. Fig. 2 shows the linear dose-response curve. Mean peak-height per ppm was 1.3 mm with the Varian Aerograph 90P and 1.9 mm with the eventually employed Packard-Becker 428 (Table 1).

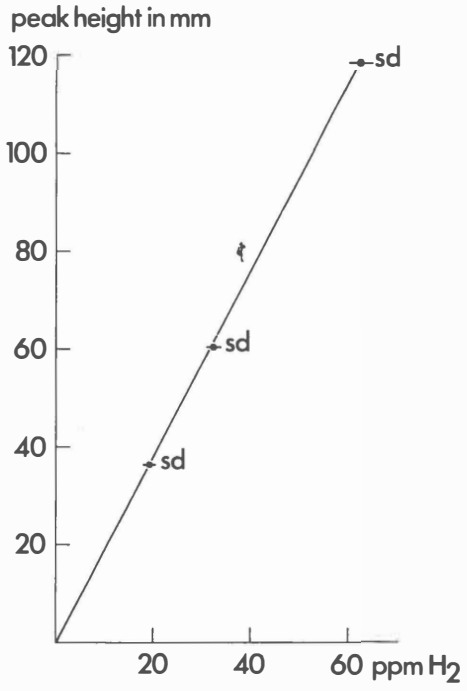

Fig. 2. Reference curve, showing linear relationship between H_2 concentration and peak height (Packard-Becker 428). For details see Table 1.

Table 1. Reproducibility of H_2 estimations from 10 determinations using precalibrated reference gases and Packard-Becker GC.

H_2 concentration ppm	SD in ppm	coefficient of variation (%)
18	0.5	3.6
32	1.1	3.3
63	2.0	3.2

Since it appears that the slope of the reference line is not always constant, it is advisable to check this every two or three days when estimations have to be done.

Volume of the injected air sample and the resultant peak height are not related in a linear way (Fig. 3) and larger samples tend to broaden the peak base. Therefore, 5 ml samples have been used with the Varian Aerograph whereas 4 ml samples are optimal with the Packard-Becker GC. 2 ml Samples may also be used but increase the standard deviation due to more pronounced influence of sampling errors.

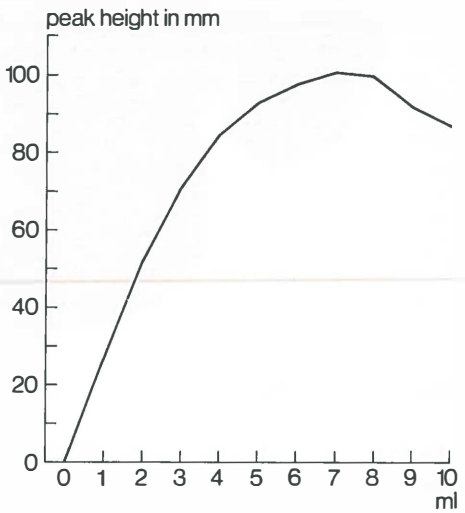

Fig. 3. Relation between injected volume and peak height with 39 ppm H_2 reference gas (Packard-Becker 428).

Sampling and storing expired air.

The principle is an open-ended collection system, consisting of a paediatric-size anaesthesia mask connected to a perspex pipe with a content of 28 ml. The infant inspires through a lateral porthole in the face-mask and expires into the pipe, at the same time expelling the room air (dead space) from the system. The collection pipe is assumed to be filled when the luminal wall shows condensation of water. This sampler is described in detail in 3.B. Eventually, a simplified modification has been constructed by C.S. Mansvelder (Medical Faculty, Free University, Amsterdam) with a straight collection pipe supplied with commercial metal gas-valves (Fig. 4).

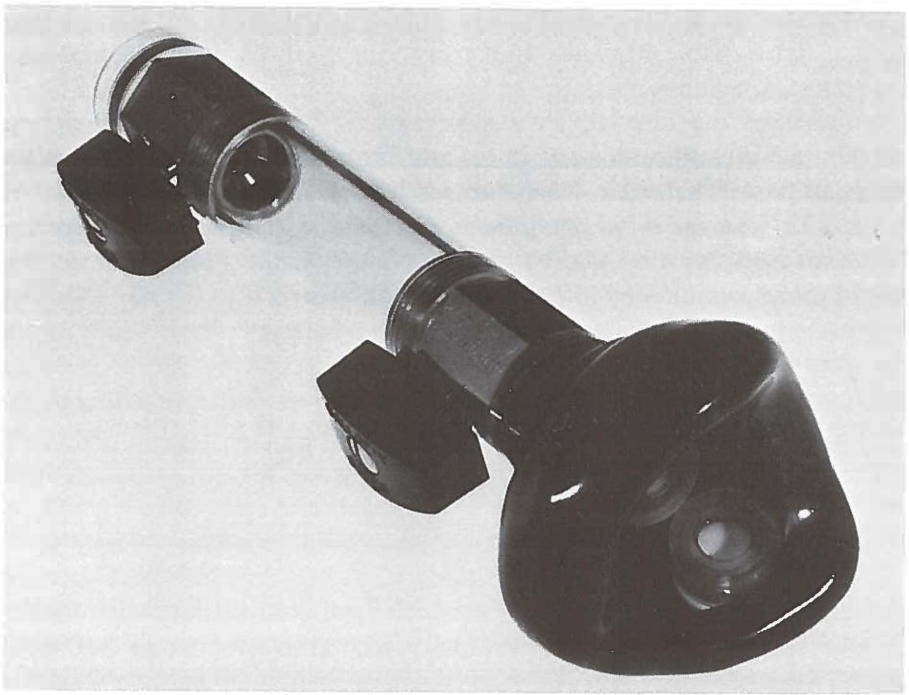

Fig. 4. Simplified modification of the collection device, constructed by C.S. Mansvelder.

Application of both types in 110 one week old newborns (chapter 8) showed that the procedure is easy to perform with minimal discomfort to the baby. This contrasts favourably with the nasal tube technique, making 80% of the infants cry and requiring assistance to immobilize the infant (Barr, 1979, personal communication). For use in older children, the mask is removed and the child expires by mouth directly into the collection pipe.

Expired air samples can be analysed directly after collection or stored in vacutainers, allowing for transport and mailing. In variance to the procedure described in 3.B, vacutainers are no longer supplied with soda-lime and silicagel since we did not notice a shorter life time of the column without it.

Loss of H_2 due to sampling and storing

Commercially available 14 ml vacutainers contain about 4 ml of air, causing a dilution of H_2 concentration of 29%. Additional evacuation of

the vacutainers as described in 3.B reduces this loss to 6.7%. Combination of sampling and storing procedures causes a loss of 18% (data derived from serial estimations with reference gases).

This loss does not affect discrimination between normal and abnormal H_2 excretion when the latter is assumed to be present if total H_2 excretion exceeds 20 ppm and normal subjects excrete less than 10 ppm H_2 (7 ± 2 ppm).

The normal value in fasting subjects should be equal to the H_2 concentration in room air. If determined gas-chromatographically, however, a too high value is obtained since outside air only contains 0.5 ppm H_2. The higher values found in gaschromatography may be attributed to summation of traces of other light gases such as helium and neon which cannot sufficiently be separated from the H_2 peak.

Differences between duplicate samples and comparison with the rebreathing technique

The nature of the sampling procedure, collecting only a part of one or two expirations, can be expected to produce different concentrations of H_2 between duplicate samples, due to the factor of anatomical dead space, frequency of respiration and the moment in the expiration phase where most of the sample has been derived from. Apart from these physiological variables, it is not known whether the excretion of H_2 remains constant in two or three subsequent expirations and results of end-expiratory air sampling suggest that this is not the case (Metz, 1976; Dolmans 1978).

In order to have some idea of the magnitude of these differences, 30 subsequent duplicate samples of the baby described in chapter 8 with H_2 concentrations ranging from 8-78 ppm (mean 28) have been analysed. Correlation between duplicate samples was statistically significant ($r = 0.93$), mean standard deviation 4 ppm and mean coefficient of variation between duplicate samples was 15%.

Additonally, 100 duplicate samples from the first 25 infants with abnormal H_2 excretion in the cross-sectional study (chapter 8) with H_2 concentrations between 8-160 ppm (mean 31) showed significant correlation ($r = 0.88$), mean standard deviation was 8 ppm and coefficient of variation 27%.

Compared to the method of end-expiratory sampling of air as used in adults, reproducibility of duplicate samples is less in our method. The

coefficient of variation between duplicate samples reported by Metz (1976) was 18%, by Dolmans (1978) 16.8 ± 9.9%. These obviously unavoidable differences between duplicate samples give rise to two questions: 1) does this affect the discrimination between normal and abnormal H_2 production, i.e. between absorbers and malabsorbers of a particular sugar and 2) does it affect the semi-quantitative nature of the breath H_2 test as has been obtained with the rebreathing procedure?

The advantage of filling a gas bag with multiple (rebreathed) expirations is that fluctuations in H_2 concentration occurring between expirations and during a single expiration are smoothed away. This method also allows to calculate H_2 excretion in terms of production in ml H_2 per minute and this has been shown to correlate well with the amount of non-absorbed sugar (Levitt, 1969).

Therefore, an answer to the above questions may be provided by comparing the results of the interval mixed-expired-air method with those of the rebreathing technique, applying both procedures simultaneously to the same subject. In doing this, it appeared that comparability was best when the higher value of the duplicate mixed-expired-air sample was used (Fig. 5).

The discriminatory value of the interval mixed-expired-air method in 22 lactose-intolerant children undergoing both methods simultaneously, was 100%.

The question about the semi-quantitative nature of the mixed-expired-air method is more difficult to answer since we studied dose-response relationship in a few patients only (chapter 6, Fig. 1). Evidence for a relationship between the amount of non-absorbed sugar and peak H_2 concentrations found in mixed-expired-air are: a) clinical symptoms are related to higher values of H_2 concentrations whereas rises between 10-20 ppm have not been accompanied by clinical symptoms in most children (chapter 4), b) sensitivity for the detection of small amounts of non-absorbed sugar, such as in the early phase of the lactose tolerance test in malabsorbers, is comparable to and often higher than with the rebreathing method. Initial abnormal rise in H_2 concentration was found to occur at the same time in 13 out of 22 (59%), preceded that of the rebreathing method in 6 out of 22 (27%) and lagged behind in 3 out of 22 (14%), c) correlation between H_2 concentrations of paired samples of both methods was 0.85 (chapter 3.B, Fig. 2). However, the correlation coefficient was lower in a larger number of paired samples. Though comparison of both methods reveals excellent discriminatory value, proving the validity of the interval-mixed-expiratory air method as a reliable

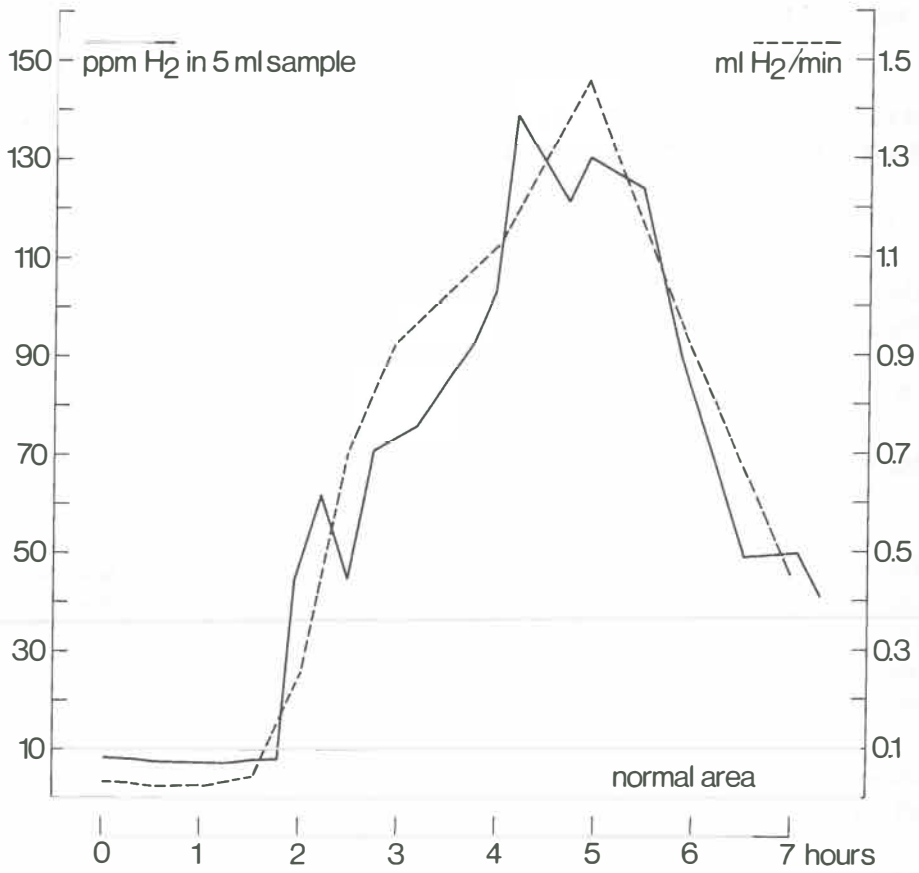

Fig. 5. Comparison of H_2 excretion as estimated with the rebreathing method and with the mixed expired air procedure, performed simultaneously in a normal adult after ingestion of lactulose (40 ml Duphalac).
– – – – – : rebreathing method, expressed in ml H_2 per minute;
——— : interval mixed expired breath method, expressed as concentration of samples.

index for sugar malabsorption in paediatric patients, its semi-quantitative quality may still be argued despite the above given evidence. It may be speculated however that comparison of these entirely different techniques can affect the results obtained with the mixed-expired-breath test.

Therefore, a more appropriate approach to test the semi-quantitative nature of our method will be the study of dose-response relationship in a number of children after administration of different amounts of the non-absorbable sugar lactulose. Apart from these considerations, the mixed or total expiratory air method provides a reliable test for clinical use (chapter 4, 5, 6) and is easy to perform in infants and very young children as well as in older children (chapter 3.B).

Chapter 3.B

HYDROGEN BREATH TEST IN INFANTS AND CHILDREN: SAMPLING AND STORING EXPIRED AIR

A.C. DOUWES, J. FERNANDES and W. RIETVELD

Division of Paediatric Gastroenterology, Sophia Children's Hospital, Erasmus University, Rotterdam (The Netherlands)

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Introduction

The measurement of hydrogen (H_2) in expired air is a sensitive method for the detection of sugar malabsorption, the gut lumen being the only site of origin (1, 5). In adults the breath H_2 test is now well established (7). Our experience over the past two years, using the rebreathing method adapted to children, shows that this method is superior to the conventional methods in detecting disaccharide malabsorption.

However, the main drawback of a rebreathing system is that it requires the cooperation of the child and is therefore not suitable for children under the age of four years.

Determination of H_2 in end-expiratory air (EAA) seems to be a reliable alternative but requires intubation of the oropharynx (6). We therefore decided to estimate H_2 in total expiratory air (TEA). For this purpose a simple sampling device was designed which we describe here.

In addition a technique has been developed for transport and storage of the collected expired air.

Materials and methods

5-ml Samples of TEA are injected into a Varian Aerograph 90P with TC detector at 100 °C, filament current 130 mA, a 2-m stainless steel column with i.d. 4 mm, packed with mol sieve 5 Å, carrier gas Argon at 17 ml/min, oven temperature 35 °C. In this way it is possible to detect

amounts of H_2 as low as 5 ppm. The sampling device (Fig. 1) consists of a curved perspex tube with an anaesthetic mask of appropriate size at the proximal end. The mask has a lateral porthole with a low resistance inlet valve. The portion of the tube which fits into the mask also has a low resistance one-way valve, the other end is open. The tube is fitted with 2 gastight stopcocks which close off a volume of approximately

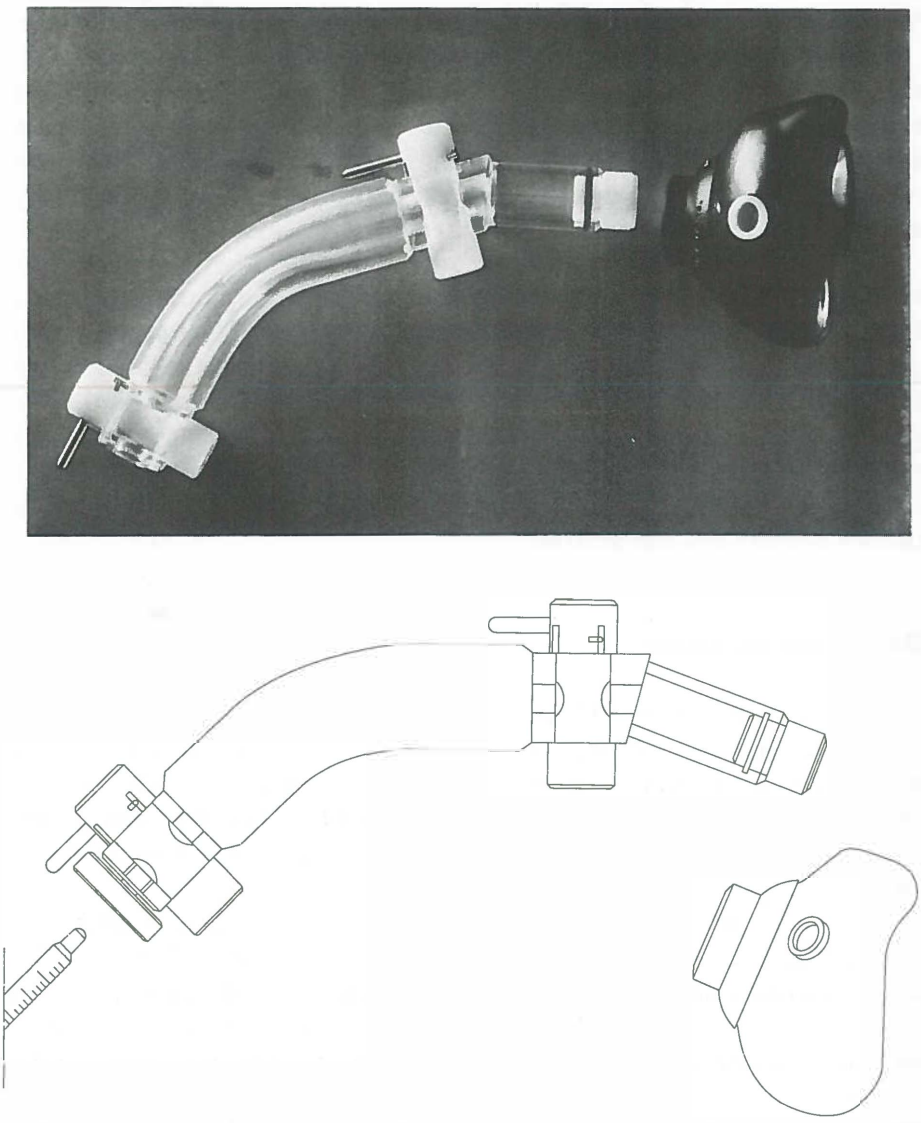

Fig. 1A. Construction of sampling device. This figure has been added to the original publication.

25 ml. The mask is placed over the nose and mouth of the child while it sits on the mother's lap. The expired air enters the tube, as can be seen by the condensation of water on the luminal wall. When the condensation reaches the distal end after 2-4 expirations, both stopcocks are closed. The sampler is taken from the child's face and a connection piece is placed on the distal end which allows aspiration of a sample, using a gas-tight 20 ml syringe (Hamilton). Since considerable sampling error may occur, a second collection is done and the higher H_2 value is used.

The breath samples are collected before and ½-hourly after sugar administration for 2½ hours and injected into specially prepared rubber sealed glass tubes (Vacutainer). The tubes are half filled with sodalime and silica gel to prevent deterioration of the column from CO_2 and water. A vacuum of 1 mm Hg is previously created in these tubes by aspiration through a 27 G needle connected with a vacuum pump.

Each tube is filled with a TEA sample so that the pressure in the tube is slightly higher than the atmospheric pressure in order to prevent inflow of surrounding air in case of leakage. The complete procedure of sampling plus storing causes a systematic loss of 20% of H_2, as estimated from serial determinations with reference gases. To correct for this H_2 loss, reference gases are led through the sampler and stored in the same manner so as to compare the appropriate reference points at the time of estimation. There is no additional loss of H_2 during a storage period of two months in a refrigerator.

Results and discussion

In order to verify the TEA technique, the results were compared with the rebreathing method. H_2 was measured in 33 pairs of samples by both techniques in five lactose intolerant children ranging from 4 to 11 years old, before and after a lactose load of 2 g/kg bodyweight and in one normal adult before and after ingestion of 20 g of the nonabsorbable sugar lactulose.

TEA was sampled either immediately before or immediately after each rebreathing period of 3 min. Fig. 2 shows the correlation between TEA determinations and those of the rebreathing method (ppm per 5 ml sample). Fig. 3 compares the results of both methods in one child during the same lactose tolerance test. In conclusion, H_2 concentrations in total expiratory air are much lower than those obtained from the rebreathing method and duplicate values are somewhat variable. However, both me-

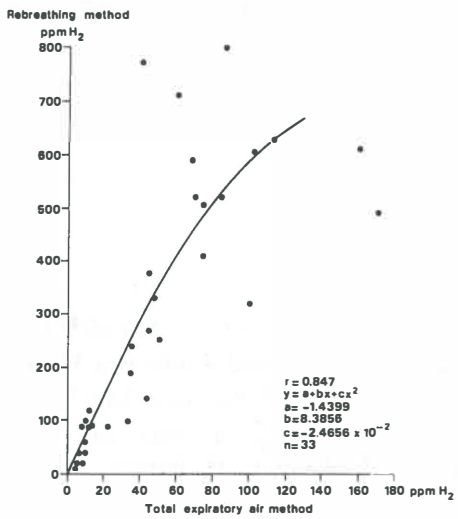

Fig. 2. Correlation between H_2 measured by the rebreathing method and H_2 in total expiratory air (TEA).

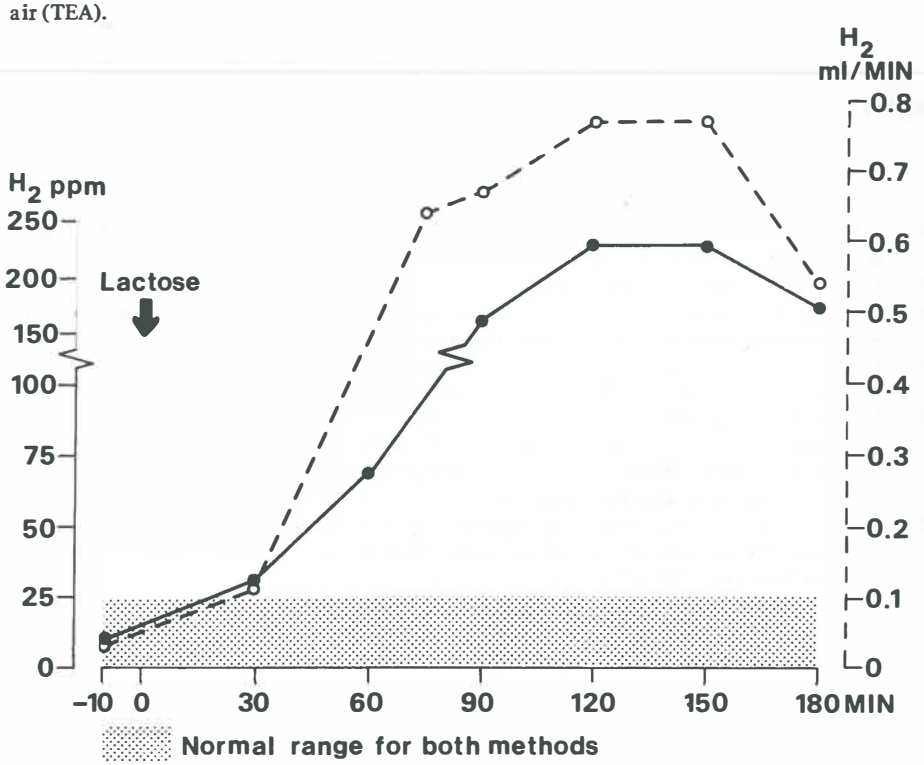

Fig. 3. Relation of the results of both methods during a lactose tolerance test in a single, lactose intolerant patient.
●——● TEA method (ppm/5 ml); ●- - - -● rebreathing method (ml H_2 expired per min).

thods are, in our hands, equally reliable in detecting sugar malabsorption. Thus, H_2 estimation in total expiratory air offers a very easy means for the detection of carbohydrate malabsorption in infants and young children.

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References

1. Calloway, D.H. and Murphy, E.I. The use of expired air to measure intestinal gas formation. Ann. N.Y. Acad. Sci. 150, 82 (1968).
2. Calloway, D.H., Murphy, E.L. and Bauer, D. Determination of lactose intolerance by breath analysis. Am. J. Digest. Dis., New Series, 14, 811 (1969).
3. Dolmans, W. Thesis, Nijmegen (1978).
4. Gearhart, H.L., Bose, D.P., Smith, C.A. and Morrison, R.D. Determination of lactose malabsorption by breath analysis with gas chromatography. Anal. Chem., 48, 393 (1976).
5. Levitt, M.D. and Donaldson, R.M. Use of respiratory hydrogen to detect carbohydrate malabsorption. J. Lab. Clin. Med. 75, 937 (1970).
6. Maffei, H.V.L., Metz, G.L. and Jenkins, D.J.A. Hydrogen breath test: adaptation of a simple technique to infants and children. Lancet, i, 1110 (1976).
7. Metz, G.L., Jenkins, D.J.A., Peters, T.J., Newman, A. and Blendis, L.M. Breath hydrogen as a diagnostic method for hypolactasia. Lancet, i, 1155 (1975).
8. Metz, G., Gasull, M.A., Leeds, A.R., Blendis, L.M. and Jenkins, D.J.A. A simple method of measuring breath hydrogen in carbohydrate malabsorption by end expiratory sampling. Clin. Sci. Mol. Med., 50, 327 (1976).

Chapter 4

IMPROVED ACCURACY OF LACTOSE TOLERANCE TEST IN CHILDREN, USING EXPIRED H_2 MEASUREMENT

A.C. DOUWES, J. FERNANDES and H.J. DEGENHART

From the Department of Paediatrics, Sophia Children's Hospital and Neonatal Unit, Academic Hospital of the Erasmus University, Rotterdam

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SUMMARY

Expired hydrogen and blood glucose were measured during an oral lactose tolerance test in 163 children aged between 9 months and 14 years. Lactose malabsorption, defined as an abnormal increase in expired H_2 during a lactose tolerance test, was found in 54 children. Of these, 30 were found to be lactose intolerant as the increased expired H_2 was accompanied by clinical symptoms. The other 109 children, in whom there was no rise in expired H_2 were assumed to have normal lactose absorption. In children with lactose intolerance the increase in expired H_2 tended to occur earlier after lactose ingestion than in children with malabsorption. The mean value of the rise in blood glucose was 2.4 mmol/l (43 mg/100 ml) in the lactose-tolerant children and 1.0 mmol/l (18 mg/100 ml) in the lactose-intolerant ones. Although this difference is significant ($P < 0.001$), the rise in blood glucose in predicting the correct diagnosis, was wrong in 13% of cases in the lactose-tolerant group, and wrong in 37% in the lactose-intolerant group (95% confidence limits 9-19% and 22-53% respectively). It is concluded that a rise in blood glucose, whether or not of more than 1.2 mmol/l (22 mg/100 ml) is of little help in differentiating lactose tolerance from intolerance.

Lactose malabsorption is usually diagnosed by the failure of blood glucose to rise normally above the fasting level after an oral lactose tolerance test (LTT). However a subnormal rise in glucose (Δ-glucose) is an imperfect indicator of lactose malabsorption, as it tells us the portion

of lactose which is absorbed, not the portion unabsorbed which is what causes the clinical symptoms. Apart from this, many factors known to influence the blood glucose level render the diagnostic value of Δ-glucose doubtful (Krasilnikoff et al. 1975; Garza and Scrimshaw, 1976; Harrison and Walker-Smith, 1977). It is therefore not surprising that there is no agreement about the value for Δ-glucose diagnostic of lactose malabsorption. It has recently been found that the LTT is interpreted more accurately by measuring expired hydrogen than by Δ-glucose, both in adults (Calloway et al. 1969; Levitt, 1969; Newcomer et al. 1975) and children (Maffei et al. 1977; Fernandes et al. 1978).

Using a procedure for H_2 determination, adapted for children and infants (Douwes et al. 1978; Fernandes et al. 1978), we have evaluated the diagnostic value of Δ-glucose in relation to expired H_2 and to clinical symptoms after an oral LTT.

Patients and methods

An LTT was performed on 163 patients ranging in age from 9 months to 14 years. Most had a history of chronic or recurrent diarrhoea and/or unexplained abdominal pain, with or without bloating. Some patients were examined because of inflammatory bowel disease or cystic fibrosis, conditions known to have an increased frequency of lactase deficiency.

Lactose (2 g/kg, maximum 50 g, 20% solution) was given orally at 9 a.m. after an overnight fast. Capillary blood was taken by finger prick at −15, 0, 15, 30, 60, 90, 120 and 150 minutes for blood glucose estimation. The mean of the glucose levels at − 15 and 0 minutes was taken as the fasting level. A Δ-glucose of $\geqslant$ 1.2 mmol/l was considered to be normal.

H_2 was measured every 30 min during a period of 180 minutes. In children aged 4 years or older, expired H_2 could be measured with a rebreathing system (Fernandes et al. 1978). An excretion $\geqslant$ 0.1 ml H_2/min was considered abnormal. In younger children and infants expired air was collected from 2 or 3 breaths (Douwes et al. 1978). An increase of $\geqslant$ 10 ppm H_2 above the fasting value was considered abnormal. In 48 children both methods were applied and the results were found concordant positive in 14, concordant negative in 32, and discrepant in 2. The last result was because one valve of the sampler was found to leak.

Lactose *malabsorption* was diagnosed on one criterion: an abnormal increase of expired H_2 during an LTT. Lactose *intolerance* was diagnosed

on two criteria: an abnormal increase of expired H_2, and abdominal pain and/or diarrhoea after the ingestion of lactose.

The patient was considered lactose tolerant if no rise in H_2 excretion could be detected.

Results

In 163 oral LTT's, both expired H_2 and blood glucose concentrations were measured. No increase in expired H_2 could be detected in 109 children, and these were considered to be lactose tolerant. An abnormal rise of H_2, indicating some degree of malabsorption, was found in 54 patients, among whom symptoms were absent in 24 and present in 30. The latter were considered to be lactose intolerant. Final diagnoses of this group are listed in Table 1.

Table 1. Final diagnosis in 30 lactose intolerant children

Diagnosis	n	Proved lactase deficiency*
Lactase deficiency		
Genetic	16	3
Secondary	9	4
Coeliac disease	2	1
Cystic fibrosis	1	
Short bowel	1	
Crohn's disease	1	1

*Proved by enzyme assay (the other patients were not biopsied).

The presence of absence of symptoms was recorded in 45 of the 109 patients who had no rise in H_2 production after an oral LTT: 6 of these complained of symptoms. Δ-Glucose of 109 lactose-tolerant patients and 54 lactose malabsorbers is shown in Fig. 1A. Δ-Glucose of 109 lactose-tolerant and 30 lactose-intolerant patients is shown in Fig. 1B.

The mean values for Δ-glucose of the patients with lactose malabsorption without clinical symptoms (group 1), lactose intolerance (group 1A), and normal lactose absorption (group 2) were 1.4, 1.0, and 2.4 mmol/l, respectively. The mean values were significantly different between groups 1A and 2 and between groups 1 and 2 ($P < 0.001$), but not between

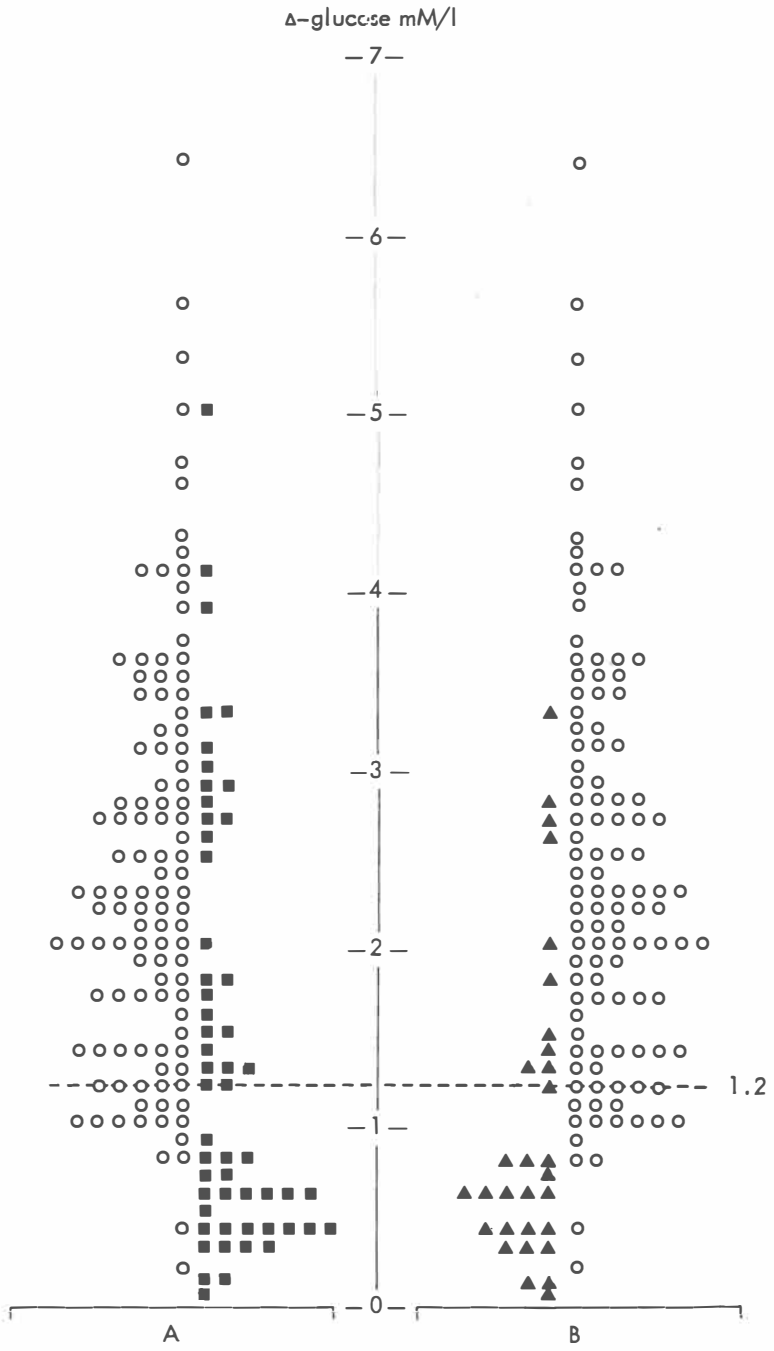

Fig. 1. Δ-Glucose in 163 children during a lactose tolerance test.
○ = normal absorption
■ = lactose malabsorption
▲ = lactose intolerance

groups 1 and 1A. However, a wide overlap both above and below the arbitrarily chosen Δ-glucose of 1.2 mmol/l as the lowest normal value is apparent from Fig. 1. Δ-Glucose failed to indicate the correct condition in half the children in group 1 (lactose malabsorption), 37% of group 1A (lactose intolerance), and 13% of group 2 (normal lactose absorption) (Table 2). The time interval between lactose administration and an abnormal increase in expired H_2 in patients with lactose malabsorption and the subgroup with additional lactose-intolerance is shown in Fig. 2. There is a tendency for a shorter time lag in the intolerant group, but both groups overlapped.

Table 2. Δ-Glucose (mmol/l) during a lactose tolerance test

Δ-Glucose	Lactose tolerance* (n = 109)	Lactose malabsorption† (n = 54)	Lactose intolerance‡ (n = 30)
Mean	2.43	1.43	1.02
Range	0.2-6.4	0.0-5.0	0-3.3
SD	1.17	1.20	0.89
False %	12.8	50	36.7
95% confidence limits	8.8-18.7	38.1-61.9	22.1-53.3

*Expired H_2 not increased; † increased expired H_2 ; ‡ increased expired H_2 plus abdominal symptoms.

Discussion

It has been emphasised that the peak rise in blood glucose (Δ-glucose) during an oral LTT can be influenced by factors other than hydrolysis and absorption of the sugar. These factors are of intestinal (peristalsis) and extraintestinal origin (normal or under use of glucose) (Krasilnikoff et al. 1975; Garza and Scrimshaw, 1976; Harrison and Walker-Smith, 1977). It is therefore not surprising that Δ-glucose after lactose ingestion differentiates poorly between normal lactose absorption and lactose malabsorption (James, 1972; Harrison and Walker-Smith, 1977), lactose intolerance (Garza and Scrimshaw, 1976; Harrison and Walker-Smith, 1977), and lactase deficiency (James, 1972; Krasilnikoff et al., 1975; Newcomer et al., 1975).

Excess of reducing substances in the stool, with high lactic acid content and low pH, if present, are of great help in this differentiation (James, 1972; Harrison and Walker-Smith, 1977), but only a few lactose-intolerant patients show these abnormalities. In the present study 2 of 11 stools examined showed them.

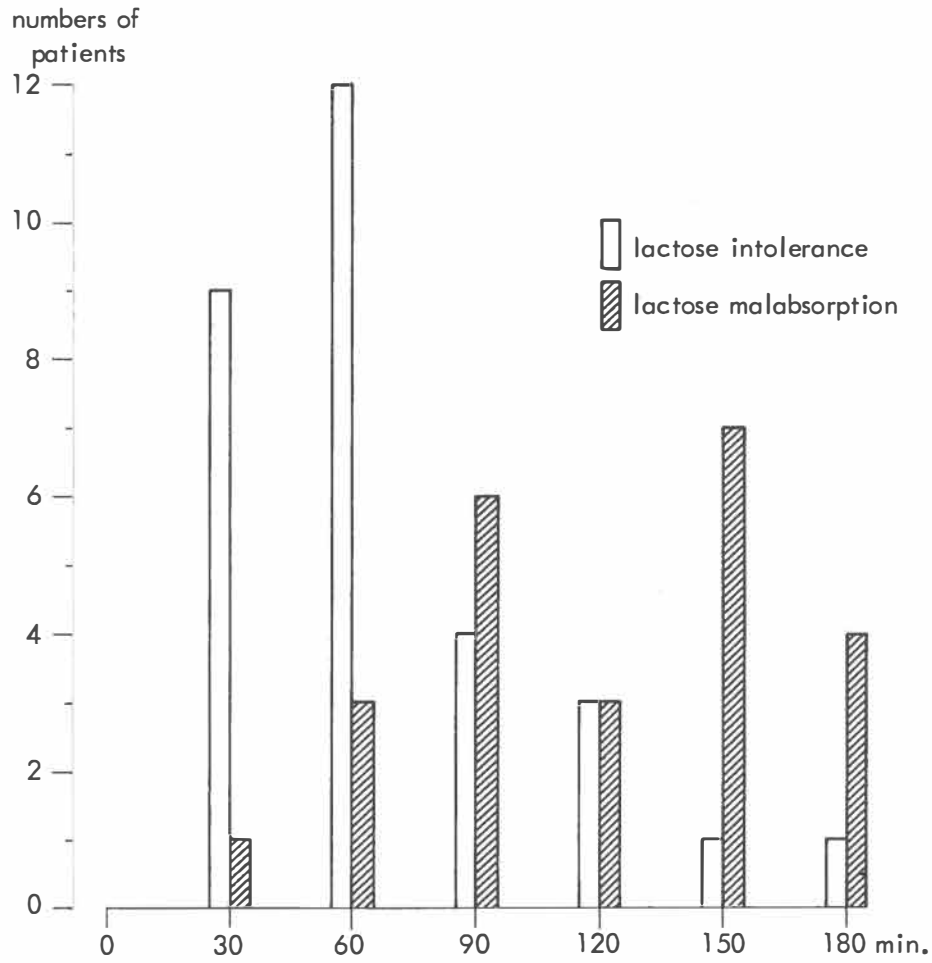

Fig. 2. Time lag between lactose ingestion and abnormal increase of expired H_2 in patients with lactose malabsorption and those with lactose intolerance.

The fact that lactose malabsorption can be detected by increased expired H_2 originating from bacterial fermentation of lactose residue in the colon, is an important contribution to the diagnosis. Recent experience shows that the method is accurate and sensitive (Levitt, 1969; Newcomer et al., 1975), and can be adapted easily for children and infants (Maffei et al., 1977; Douwes et al., 1978; Fernandes et al., 1978) Taking normal or increased H_2 excretion as a reflection of normal or poor absorption of lactose, this parameter has been used to evaluate Δ-glucose during an

LTT in children (Maffei et al., 1977). Maffei et al. measured expired H_2 in 23 children with chronic diarrhoea and in 4 controls during LTT. They found no correlation between the H_2 production and blood glucose rise, but blood glucose was measured in only 7 of the H_2-producing patients and in 2 controls. We agree with their conclusions, but find the number of observations too small.

In the present study, of 163 children 54 showed increased H_2 during an LTT. Of these, 27 showed a normal Δ-glucose ($\geqslant$ 1.2 mmol/l) (Fig. 1). Thus half of the lactose malabsorbers had a false normal Δ-glucose. 30 of the 54 malabsorbers were diagnosed as being intolerant because they had clinical symptoms; of these 11 showed a normal Δ-glucose. This amounts to 37% with a falsely normal Δ-glucose. 109 patients showed no increased H_2 excretion and were therefore assumed to be lactose tolerant. Of these 14 had a Δ-glucose $<$ 1.2 mmol/l, which amounts to an error of 13%. Extrapolating these results to larger populations, the incidence of a false normal Δ-glucose would have been 38-62% for lactose malabsorption, and 22-53% for lactose intolerance, and the incidence of a false abnormal Δ-glucose 9-19% for lactose tolerance (Table 2). Thus, Δ-glucose during the LTT, appears to be an unreliable indicator of lactose malabsorption and lactose intolerance, and should be replaced by more reliable methods – such as the H_2 test.

Although we did not aim to use the H_2 test for differentiating lactose malabsorption from lactose intolerance we confirmed the observation of Maffei et al. (1977) that the increase in H_2 excretion tends to be early in lactose-intolerant patients (Fig. 2). However, the time lag between lactose administration and an increase in H_2 excretion overlaps in both conditions. Furthermore, we noticed that some of the patients with an early rise of expired H_2 and biopsy-proved lactase deficiency, did not develop symptoms during or shortly after the test but did do so on a repeat test. These facts suggest that there is a gradual transition from lactose malabsorption to lactose intolerance.

Acknowledgements

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References

Calloway, D.H., Murphy, E.L. and Bauer, D. Determination of lactose intolerance by breath analysis. American Journal of Digestive Diseases, New Series, 14, 811-815 (1969).

Douwes, A.C., Fernandes, J. and Rietveld, W. Hydrogen breath test in infants and children: sampling and storing expired air. Clinica Chimica Acta, 82, 293-296 (1978).

Fernandes, J., Vos, C.E., Douwes, A.C., Slotema, D. and Degenhart, H.J. Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. American Journal of Clinical Nutrition, 31, 597-602. (1978)

Garza, C. and Scrimshaw, N.S. Relationship of lactose intolerance to milk intolerance in young children. American Journal of Clinical Nutrition, 29, 192-196 (1976).

Harrison, M. and Walker-Smith, J.A. Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity, and lactose tolerance tests. Gut, 18, 48-52 (1977)

James, W.P.T. Comparison of three methods used in the assessment of carbohydrate absorption in malnourished children. Archives of Disease in Childhood, 47, 531-536 (1972).

Krasilnikoff, P.A., Gudmand-Høyer, E. and Moltke, H.H. Diagnostic value of disaccharide tolerance tests in children. Acta Paediatrica Scandinavica, 64, 693-698 (1975).

Levitt, M.D. Production and excretion of hydrogen gas in man. New England Journal of Medicine, 281, 122-127 (1969).

Maffei, H.V.L., Metz. G., Bampoe, V., Shiner, M. and Brook. C.G.D. Lactose intolerance, detected by the hydrogen breath test, in infants and children with chronic diarrhoea. Archives of Disease in Childhood, 52, 766-771 (1977).

Newcomer, A.D., McGill, D.B., Thomas, P.J. and Hofmann, A.F. Prospective comparison of indirect methods for detecting lactase deficiency. New England Journal of Medicine, 293, 1232-1236 (1975).

Chapter 5

DIAGNOSTIC VALUE OF SUCROSE TOLERANCE TEST IN CHILDREN EVALUATED BY BREATH HYDROGEN MEASUREMENT

A.C. DOUWES, J. FERNANDES, and A.A. JONGBLOED

From the Department of Paediatrics, Sophia Children's Hospital and Neonatal Unit, Academic Hospital of the Erasmus University, Rotterdam.

Abstract

Douwes, A.C., Fernandes, J., Jongbloed, A.A. (Sophia Children's Hospital of the Erasmus University, Rotterdam, The Netherlands).
Diagnostic value of sucrose tolerance test in children, evaluated by breath hydrogen measurement.
An oral sucrose tolerance test was performed in a group of 103 children, aged between 3 months and 15 years because of episodic diarrhea and/or abdominal pains. Sucrose malabsorption defined as an abnormal increase in expired hydrogen, was found in only 3 children who suffered from congenital sucrase-isomaltase deficiency. This 1% incidence of sucrose malabsorption was lower than the incidence of lactose malabsorption found in this group (33%). Mean rise in blood glucose during the sucrose test was higher (3.4 ± 1.4 vs. 2.4 ± 1.2 mmol/l, $p < 0.0001$) and the occurence of false flat blood glucose curves was lower (3% vs. 12.8%, $p < 0.05$) than during the lactose test. These findings are consistent with the higher sucrase activity in the small bowel mucosa compared to lactase. In contrast to the lactose tolerance test, sucrose tolerance test should not be used as a screening procedure for secondary disaccharidase deficiency in children.

The presence of non absorbed sugars in the gut lumen may produce diarrhea, meteorismus and abdominal pains (7, 13) and since dietary restriction of these sugars is beneficial, procedures have been developed to examine the tolerance to selected sugars. The oral lactose tolerance test (LTT) and the oral sucrose tolerance test (STT) are the most widely used procedures and paediatricians rely on flat blood glucose curves to assess the presence of malabsorption and start elimination diets. The challenge

dose and the arbitrarily chosen lowest normal value for the rise in blood glucose is the same in LTT and STT (6).

Theoretically, this may affect the diagnostic value of the STT since the activity of sucrase in the brush border is about twice as high as lactase. In order to evaluate this, we studied children and infants with intermittent diarrhea and/or abdominal pains by serial estimation of expired hydrogen (H_2) and rise in blood glucose during the STT and LTT.

Patients and methods

An STT was done in 103 patients, ages ranging from 3 months to 15 years. Most of them had episodic diarrhea and/or abdominal pains of undetermined cause, some suffered from cystic fibrosis, inflammatory bowel disease, gluten sensitive enteropathy or genetic lactase deficiency. The results of the LTT in these patients have been reported previously (4).

Sucrose 2 g/kg, maximum 50 g, 20% solution) was given orally at 9 a.m. after an overnight fast. Blood glucose was estimated in capillary blood at 0, 15, 30, 60, 90, 120 and 150 minutes. A rise in blood glucose above the fasting level (Δ-glucose) of $\geqslant 1.2$ mmol/l was considered normal. H_2 concentration was measured every 30 minutes during the same period using a rebreathing method (5) in the first 43 patients (normal excretion < 0.1 ml H_2/min.) and in the remaining 60 estimated in 5 ml samples of mixed expired air (normal concentrations < 10 ppm above the fasting level) according to our recently described method (3). The technique of sampling mixed expired air is suitable for children of all ages (Fig. 1). Sucrose intolerance was diagnosed on two criteria: a) an abnormal increase of expired H_2 and b) diarrhea and/or abdominal pain after sucrose challenge. Children with sucrose intolerance were biopsied for microscopic examination of the duodenal mucosa and quantitative assay of the disaccharidases (2, 12). Disaccharidase activity of 12 sucrose tolerant children was estimated because of an abnormal LTT. A sucrase activity of $\geqslant 1.9$ μmoles sucrose split per minute per g mucosa, was considered normal (12).

Results

Of the 103 children studied, 100 had normal H_2 excretion during the STT, indicating that no sucrose malabsorption occurred. Two of 44 chil-

Fig. 1. Sampling device for mixed expired air for all ages. The anaesthetic mask with lateral low resistance inlet valve is used for infants. Older children may expire directly into the collection pipe.

dren asked for clinical symptoms during the STT however had abdominal pains and 1 was flatulent. However, since stools passed during or shortly after the test revealed normal values for pH, reducing substances and lactate, these children were considered sucrose tolerant.

The 3 children with abnormal excretion of H_2 had diarrhea and abdominal cramps but Δ-glucose was normal in 2 of them. Fig. 2 illustrates our findings in one of these. The normal H_2 excretion in one of these sucrose intolerant patients after administration of equivalent amounts of glucose and fructose demonstrated that the basic defect was in the hydrolysis of sucrose (Fig. 3). This was confirmed by assay of the small bowel disaccharidases: all 3 patients had congenital sucrase-isomaltase deficiency.

Fig. 4 shows the Δ-glucose of the 100 sucrose tolerant children and the Δ-glucose in 109 lactose tolerant children reported previously (4). The mean value for Δ-glucose in the sucrose tolerant group is significantly higher ($p < 0.0001$) than in the lactose tolerant group (Table 1).

The occurrence of false flat glucose curves in the STT and LTT groups

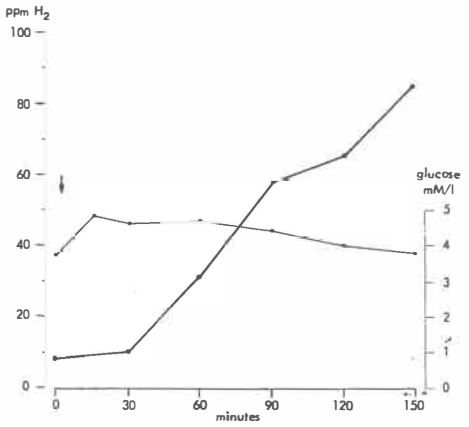

Fig. 2. H_2 excretion (●————●) and blood glucose curve (●————●) during an STT in a 5 year old boy with sucrase-isomaltase deficiency (mixed expiratory air method).

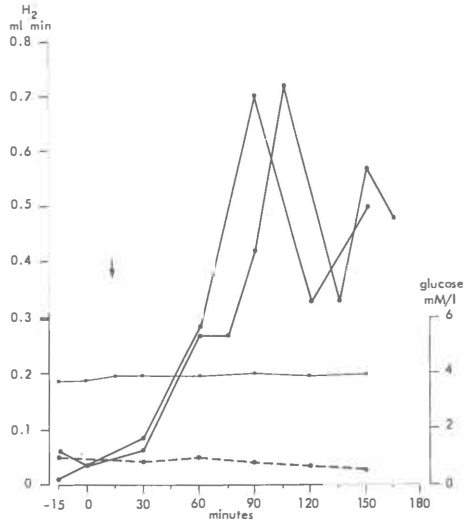

Fig. 3. H_2 excretion (●————●) and blood glucose curve (●————●) during two subsequent STT's and H_2 excretion (●--------●) after challenge with the monosaccharides of sucrose in a 6 year old boy with sucrase-isomaltase deficiency (rebreathing method).

is 3% and 12.8% respectively. Since the 95% confidence limits of these percentages do not overlap, the lower incidence of false flat blood glucose curves in the STT group is significant ($p < 0.05$).

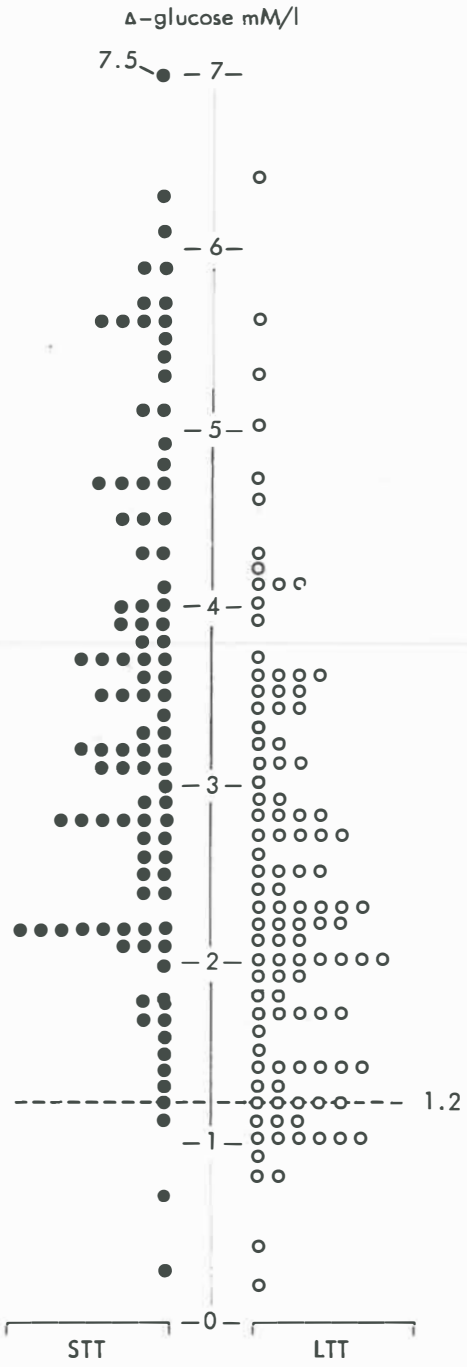

Fig. 4. Δ-Glucose in tolerant subjects (no abnormal H_2 increase) during an STT (n = 100) and during an LTT (n = 109).

Table 1. Δ-Glucose in mmol/l during lactose and sucrose tolerance test in subjects tolerant for these disaccharides.
* $p < 0.0001$ ** $p < 0.05$

Δ-Glucose	sucrose tolerant (n = 100)	lactose tolerant (n = 109)
mean and SD	3.42 ± 1.43	2.43 ± 1.17 (s)*
95% confidence limits	3.14 - 3.71	2.21 - 2.65
range	0.3 - 7.5	0.2 - 6.4
% false	3	12.8 (s) **
95 % confidence limits	1.5 - 8.0	8.8 - 18.7

Sucrase activity in the duodenal mucosa of 11 sucrose tolerant children ranged from 3.9-8.8 μmoles sucrose split per minute per g mucosa (normal ⩾ 1.9). Another sucrose tolerant child with untreated gluten sensitive enteropathy had 0.9 sucrase activity and was therefore sucrase deficient.

Discussion

There are a few reports on sucrose malabsorption (SM) and these are limited to sucrase-isomaltase deficiency (1, 10). Secondary SM is also rare and only occurs with severe enteropathy, short bowel syndrome and bacterial contamination of the small intestine (unpublished observations) and has not been reported in post gastroenteritis enteropathy. In fact, children with acute gastroenteritis can be treated by oral administration of sucrose-saline solutions (8, 11). In the present study only 3 out of 103 children (1%) appeared to have SM in contrast to 54 of 163 (33%) having lactose malabsorption (4). We did not find secondary SM. Even the patient with sucrase deficiency due to gluten sensitive enteropathy was found to be sucrose tolerant. A similar finding has been reported earlier (6).

The rare occurrence of secondary SM may be explained by the finding that the brush border sucrase activity is about twice as high as lactase (9). Furthermore, this high sucrase activity probably accounts for the low number of false flat glucose curves during an STT. Surprisingly, this has not been taken in consideration since challenge dose and definition of normal Δ-glucose are the same in STT and LTT (6). The present data show that during an STT mean Δ-glucose is higher and the chance of

finding a false flat glucose curve lower than during an LTT. This contrasts with an earlier report finding the same high incidence of false flat curves during STT and LTT, 24-33% and 23-30% respectively (6).

This may be due to the smaller number of children and absence of H_2 determinations as a reliable indicator for disaccharide malabsorption in that study. Our data indicate that the diagnostic value of STT and LTT is different in that secondary sucrase deficiency is not likely to be detected by the STT, even with the help of H_2 breath analysis. Theoretically, doubling the sucrose challenge to 4 g/kg might be helpfull but since this load is unphysiological we did not study it. We therefore recommend that the STT, in contrast to the LTT, should not be used as a routine procedure for the detection of secondary disaccharide malabsorption in children and infants. It may only be useful in children thought to have primary sucrase-isomaltase deficiency.

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References

1. Ament, M.E., Perera, D.R. & Esther, L.J. Sucrase-isomaltase deficiency, a frequently misdiagnosed disease. J. Pediatr. 83: 721, 1973.
2. Dahlquist, A.: Method of assay of intestinal disaccharidases. Analyt Chem, 7: 18, 1964.
3. Douwes, A.C., Fernandes, J. & Rietveld, W.: Hydrogen breath test in infants and children: sampling and storing expired air. Clin Chim Acta, 82: 293, 1978.
4. Douwes, A.C., Fernandes, J. & Degenhart, H.J.: Improved accuracy of lactose tolerance test in children, using expired H_2 measurement. Arch Dis Child, 53: 939, 1978.
5. Fernandes, J., Vos, C.E., Douwes, A.C., Slotema, E. & Degenhart, H.J.: Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. Am J. Clin Nutr, 31: 597, 1978.
6. Krasilnikoff, P.A., Gudmand-Høyer, E. & Moltke, H.H.: Diagnostic value of disaccharide tolerance tests in children. Acta Paed Scand, 64: 693, 1975.
7. Launiala, K.: The mechanism of diarrhoea in congenital disaccharide malabsorption. Acta Paed Scand, 57: 425, 1968.
8. Nalin, D.R., Mata, L., Vargas, W., Loria, A.R., Levine, M.M., Cespedes, C., De, Lizano, C., Simhon, A., & Mohs, E.: Comparison of sucrose with glucose in oral therapy of infant diarrhoea. Lancet, ii: 277, 1978.
9. Newcomer, A.D., McGill, D.B.: Distribution of disaccharidase activity in the small bowel of normal and lactase-deficient subjects. Gastroenterology, 51: 481, 1966.
10. Perman, J.A., Barr, R.G. & Watkins, J.B.: Sucrose malabsorption in children: noninvasive diagnosis by interval breath hydrogen determination. J Pediatr, 93: 17, 1978.

11. Sack, D.A., Eusof, A., Merson, M.H., Black, R.E., Chowdhury, A.M.A.K., Ali, M.A., Islam, S. & Brown, K.H.: Oral hydration in rotavirus diarrhoea: a double blind comparison of sucrose with glucose electrolyte solution. Lancet, ii: 280, 1978.
12. Townley, R.R.W., Khaw, K.T. & Shwachman, H.: Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood. Pediatrics, 36: 911, 1965.
13. Weijers, H.A., Kamer, J.H. van de, Dicke, W.K. & IJsseling, J.: Diarrhoea caused by deficiency of sugar splitting enzymes. I. Acta Paediat Scand, 50: 55, 1961.

Chapter 6

INTERVAL BREATH HYDROGEN TEST IN GLUCOSE-GALACTOSE MALABSORPTION

Adriaan C. DOUWES, Micheline VAN CAILLIE-BERTRAND, John FERNANDES and Charles M.A. BIJLEVELD

Sophia Children's Hospital and Neonatal Unit, Academic Hospital of the Erasmus University, Rotterdam, The Netherlands

JEHAN F. DESJEUX
Inserm U 83, Hôpital Hérold, 75935, Paris, France

Speculation

Interval sampling of mixed expired air and estimation of hydrogen (H_2) in these samples after an oral load with monosaccharides provides a reliable, noninvasive index of monosaccharide malabsorption. A presumptive diagnosis of hereditary glucose-galactose malabsorption can be made by H_2 breath determinations after subsequent challenge with glucose, galactose and fructose. This allows for early therapeutic measures before definite confirmation of the diagnosis by more invasive and time consuming procedures is obtained. The H_2 breath test will be a useful tool for the study of sugar absorption in neonates.

Hereditary glucose-galactose malabsorption (GGM), first recognized in 1962 (8, 9) is the only primary disorder of monosaccharide absorption reported and so far 23 cases have been described in infants. Severe diarrhoea results from feeding breast milk or cow's milk formula until all sugars except fructose, are excluded from the diet.

The diagnostic methods are similar to those used in disaccharide malabsorption, i.e. oral sugar tolerance tests with estimation of blood glucose and faecal reducing substances. However, the rise in blood glucose is an unreliable index of sugar absorption whereas the absence of excess reducing substances does not exclude malabsorption (3). Confirmation of the diagnosis of GGM requires intubation studies of intestinal absorp-

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tion (12) or the use of mucosal biopsy specimens for autoradiography with ^{14}C-labeled monosaccharide (14) or quantitative ^{14}C-glucose transport studies (7).

The supply of monosaccharides to human intestinal flora results in their fermentation and the production of H_2 during in vitro studies (1). In man, glucose loading with subsequent determination of expired H_2 is used to detect bacterial overgrowth of the small bowel (13). Since in earlier clinical studies in children rebreathing systems have been used for collection of expired air (6), methods which are not suitable for children under the age of four years, no reports are available concerning the detection of monosaccharide malabsorption in childhood. The recent development of sampling end-expiratory air (11) or mixed expiratory air (4, 16) at fixed intervals after an oral carbohydrate dose, renders the application of the H_2 breath test in younger children and infants feasible, however (3, 5).

The present study shows that malabsorption of the monosaccharides glucose and galactose in an infant with hereditary GGM can be detected by H_2 measurements in mixed expiratory air samples, even after a low challenge dose. The results of H_2 breath tests are compared with clinical symptoms, measurement of pH, reduction and lactate in the stools which were passed during the test period and with the results of ^{14}C-glucose transport studies in a small bowel mucosal specimen.

Case report and methods

Patient

A female infant, second child of Turkish parents who are first cousins, was born after a normal pregnancy, birthweight 3100 g. Her 3 year old sister is healthy. Diarrhoea occurred after the first breast feedings. After 1 week she weighed 2320 g and was referred to a district hospital where, after intravenous rehydration, feeding was restarted by continuous stomach drip with diluted low lactose cow's milk formula and glucose. As she continued to pass 5-12 watery stools per day, the infant was referred to our department at age 16 days. Since she weighed only 2750 g and had severe intractable diarrhoea, total parenteral nutrition was given for 1 week resulting in complete disappearance of the diarrhoea and satisfactory weight gain. Subsequent oral administration of 8 x 10 ml dextrose 5% per day again provoked frequent loose stools with pH 6 and small amounts of reducing substances. The diagnosis of GGM was considered

and the infant was placed on a carbohydrate free diet supplemented with 5% fructose, vitamins and comminuted beef on which she thrived well. She was discharged at age 3 months weighing 3980 g and by 5½ months her weight was 7.2 kg. At this time the infant was readmitted for further examination.

Methods

The tolerance tests were initiated after an overnight fast with oral administration of glucose (0.5 and 2 g per kg), galactose (1 g per kg) and fructose (2 g per kg) in 10% solutions.

Breath samples of mixed expired air were taken with a pediatric anesthesia facemask attached to an open ended 28 ml capacity perspex tube before and at 30, 60, 90, 120 and 150 minutes after sugar administration. The collection system has been previously presented in detail (4). The infant inspires through a lateral porthole in the mask, supplied with a low resistance one-way valve and expires through a similar valve into the tube at the same time expelling the room air from the collection system. When the condensation of water on the luminal wall reaches the distal end of the tube after 2-4 expirations, the two gastight stopcocks at both ends of the tube are closed. A connection piece attached to the distal end of the perspex tube allows for aspiration of expired air with a 10 or 20 ml plastic syringe. 5 ml Samples of expired air are used for gaschromatographical analysis according to our previously described method (4). 14 ml Samples can be stored in Vacutainers (Terumo Corp. Tokio, Japan) waiting for analysis. Hydrogen concentration was determined by plotting the peak height on a reference line based on precalibrated reference gases containing 20, 60 and 200 parts pro million (ppm) H_2 in nitrogen (Air Products, Nederland B.V., Waddinxveen). The mean peak height with 60 ppm reference gas was 83 ± 2 mm with a coefficient of variation of 2.4%. Blood glucose determinations, using a standard glucose oxidase method were done in capillary blood taken by finger prick during the fructose and galactose tests at 0, 15, 30, 60, 90, 120 and 150 minutes. Jejunal biopsies were performed at the age of 5½ and 14 months, using a twin hole pediatric biopsy capsule (15). Disaccharidase activities were measured (2, 17). In vitro D-glucose transport with labeled glucose was measured in jejunal mucosal biopsy specimens. The intracellular to medium (C/M) glucose ratio was measured as described previously (7). The incubation solution was Ringer solution (Na = 140 mM/l) with 10 mM D-glucose. Glucose-flux from incubation solution to cell across the

brush-border membrane was measured on intact mucosa as previously presented (7) and the results were compared to control values from the same laboratory.

Results

Oral challenge with 0.5 g per kg glucose resulted in a moderate H_2 increase, starting at 90 minutes, maximum 37 ppm H_2 at 150 minutes. Three hours after the challenge the infant passed 1 loose stool with pH 5 and 1% reducing substances. With glucose 2 g per kg (Fig. 1) an abnormal increase of H_2 occurred after 30 minutes, maximum 183 ppm at 150 minutes and later, 6 watery stools were passed with pH 4.4, reducing substances 2% and lactate increasing from 2.2 to 11.5 mmol/kg faeces. Galactose challenge therefore, was performed with only 1 g per kg.

Expired H_2 increased abnormally after 30 minutes, maximum 93 ppm H_2 at 90 minutes. No blood glucose rise occurred and 2 loose stools were produced after the test, pH declining from 5 to 4.7, reducing substances increasing from ½ to 2% with no excess lactate.

Fructose challenge with 2 g per kg did not result in increased H_2, nor in diarrhoea but blood glucose rose with only 0.7 mmol/l. Jejunal biopsy showed pale appearance of the mucosa under the dissecting microscope, grade 0-1 atrophy in light microscopy and low activity of all disaccharidases. Since this might have resulted from the preceding tolerance tests, jejunal biopsy was repeated at age 14 months. Morphology and disaccharidase activities were normal. ^{14}C-glucose transport studies in the biopsy specimen revealed no accumulation of D-glucose against a concentration gradient: the C/M ratio was 1.34 (controls 3.97 ± 0.83). The glucose influx at the brush border membrane was much reduced: 0.9 μmol/h/cm^2 (controls: 4.4 ± 0.68).

Discussion

GGM is a rare autosomal recessive disorder characterized by severe watery and acid diarrhoea starting within a few days after birth. As in our patient, most cases occur in children from consanguinous marriages. The condition is easily misdiagnosed as lactase deficiency since most clinicians have to rely on blood glucose rise during oral sugar tolerance tests and lactose may be expected to produce a flat glucose curve whereas

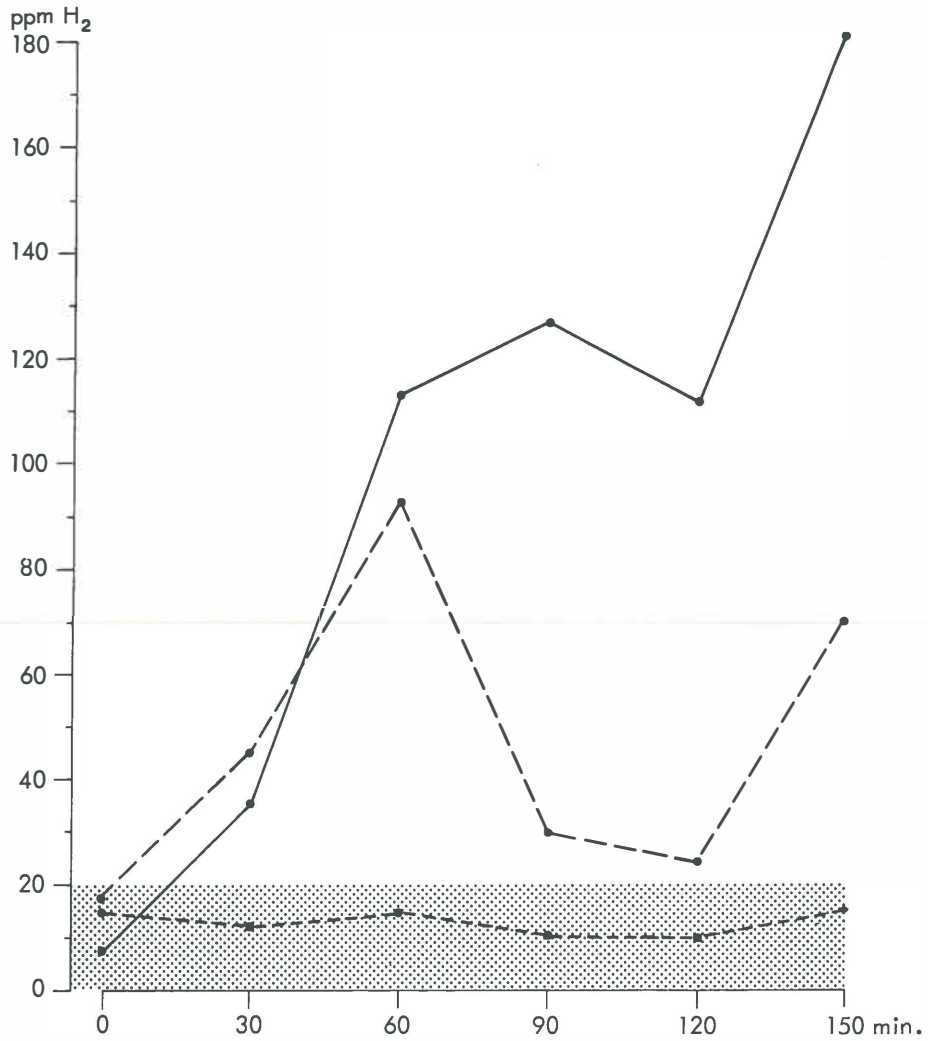

Fig. 1. H_2 excretion after oral administration of 2 g/kg glucose (●——●), 1 g/kg galactose (● – – – – –●) and 2 g/kg fructose (● - - - - - - - - - - - ●) in an infant with hereditary glucose-galactose malabsorption. Shaded area represents normal range of H_2 excretion. Note the dose related H_2 response and the similar biphasic shape of the abnormal curves. The results of the challenge with 0.5 g/kg glucose have been omitted from the figure for reasons of clarity.

a rise in blood glucose after sucrose may be derived from the fructose component. A rise in blood glucose may also be found after ingestion of glucose or galactose since 5-10% of these sugars may be absorbed in GGM (10).

Since we did not know whether H_2 is generated from fructose in amounts comparable to glucose and galactose, the infant could have a generalized monosaccharide malabsorption despite the fact that she did not show increased H_2 production after fructose. To exclude this improbability, we tested the ability of the colonic microbial flora to produce H_2 from fructose by adding 0.5 g fructose to 3-4 g freshly passed stools, homogenised in 5 ml 0.9% NaCl, incubated at 37° C, in rubber sealed, 14 ml capacity glass tubes (Vacutainer). After 3 h., H_2 production in the test tube was abundant, in contrast to the specimens without added fructose. This finding lef' us with the presumptive diagnosis of GGM which was ultimately confirmed by the ^{14}C-glucose transport studies in a specimen of small bowel mucosa.

This study shows that the interval H_2 breath test, using mixed expired air, can be used as a noninvasive procedure to detect malabsorption of monosaccharides.

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References and notes

1. Calloway, D.H., Colasito, D.J., Mathews, R.D.: Gases produced by human intestinal microflora. Nature, 212: 1238, 1966.
2. Dahlquist, A.: Method for assay of intestinal disaccharidases. Anal. Chem, 7: 18, 1964.
3. Douwes, A.C., Fernandes, J. and Degenhart, H.J.: Improved accuracy of lactose tolerance test in children, using expired H_2 measurement. Arch. Dis. Child., 53: 939, 1978.
4. Douwes, A.C., Fernandes, J. and Rietveld, W.: Hydrogen breath test in infants and children: sampling and storing expired air. Clin. Chim. Acta, 82: 293, 1978.
5. Douwes, A.C., Fernandes, J. and Jongbloed, A.A.: Diagnostic value of sucrose tolerance test in children evaluated by breath hydrogen measurement. Acta Paediat. Scand., in press.
6. Fernandes, J., Vos, C.E., Douwes, A.C., Slotema, E. and Degenhart, H.J.: Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. Am. J. Clin. Nutr., 31: 597, 1978.
7. Grasset, E., Heyman, M., Dumontier, A.M. and Lestradet, H.: Possible sodium and D-glucose co-transport in isolated jejunal epithelium of children. Pediat. Res., in press.
8. Laplane, R., Polonovski, C., Etienne, M., Debray, P., Lods, J.C. and Pisarro, B.: L'intolérance aux sucres à transfert-intestinal actif. Les rapports avec l'intolérance au lactose et le syndrome coeliaque. Arch. Franc. Pediat., 19: 895, 1962.
9. Lindquist, B. and Meeuwisse, G.: Chronic diarrhoea caused by monosaccharide malabsorption. Acta Paediat. Scand., 52: 674, 1962.

10. Linneweh, F., Schaumlöffel, E. and Barthelmai, W.: Angeborene Glucose und Galactose Malabsorption. Klin. Wschr., 43: 405, 1965.
11. Maffei, H.V.L., Metz, G., Bampoe, V., Shiner, M. and Brook, C.G.D.: Lactose intolerance, detected by the hydrogen breath test, in infants and children with chronic diarrhoea. Arch. Dis. Child., 52: 766, 1977.
12. Meeuwisse, G. and Melin, K.: Glucose-galactose malabsorption. A clinical study of 6 cases. Acta Paediat. Scand. suppl. 188: 3, 1969.
13. Metz, G., Drasar, B.S., Gasull, M.A., Jenkins, D.J.A., Blendis, L.M.: Breath hydrogen test for small-intestinal bacterial colonisation. Lancet, i: 668, 1976.
14. Schneider, A.J., Kinter, W.B. and Stirling, C.E.: Glucose-galactose malabsorption. Report of a case with autoradiographic studies of mucosal biopsy. New Eng. J. Med., 274: 305, 1969.
15. Sebus, J., Fernandes, J. and Bult, J.A., van der: A new twin hole capsule for peroral intestinal biopsy in children. Digestion, 1: 193, 1968.
16. Solomons, N.W. and Viteri, F.: Development of an interval sampling hydrogen (H_2) breath test for carbohydrate malabsorption in children: evidence for a a circadian pattern of breath H_2 concentration. Pediat. Res., 12: 816, 1978.
17. Townley, R.R., Khaw, K.T. and Shwachman, H.: Quantitative assay of disaccharidase activities of small bowel biopsy specimens in infancy and childhood. Pediatrics, 36: 911, 1965.

Chapter 7

BREATH HYDROGEN STARCH TOLERANCE TEST IN CYSTIC FIBROSIS

A.C. DOUWES, F. DE BIÉVILLE and J. FERNANDES

Abstract

Douwes, A.C., de Biéville, F. and Fernandes, J. (Sophia Children's Hospital and Neonatal Unit, Academic Hospital of the Erasmus University, Rotterdam, The Netherlands).
Breath hydrogen starch tolerance test in cystic fibrosis.
Hydrogen (H_2) excretion before and after a testmeal with amylopectin has been estimated in 14 children suffering from cystic fibrosis (CF). Only 2 of them showed a moderate rise in H_2, indicating some degree of malabsorption. Fasting H_2 levels were abnormal in 65% (15/23) of CF patients, values ranging from 11-70 ppm (mean 24 ± 16 ppm) H_2. In 87% (13/15) of these patients, respiratory H_2 excretion remained abnormal for several hours.

The amylopectin test, using breath H_2 estimations, does not detect pancreatic insufficiency in children with CF. The finding of abnormal fasting levels in many of these patients has not been reported previously and may lead to erroneous interpretation of breath H_2 tolerance tests in children with CF.

Starch is a mixture of two different polyglucoses, amylose and amylopectin. Amylose has a linear structure (1,4α-glucosidic bonds), amylopectin has a branched structure since 4% of the bonds are 1,6α-glucosidic bonds. In infants, duodenal digestion of starch is incomplete as a consequence of the low levels of α-amylase activity (1). In infants of 4-7 months old however, dietary starch is completely utilized and only trace amounts are excreted in the stools, coefficient of absorption being > 99% (2). Deficiency of pancreatic amylase may cause starch malabsorption as has been shown in adults with pancreatitis or pancreatic carcinoma (12). Since bacterial fermentation of starch gives rise to the production of H_2 (5, 6) and this may be expected to be found in children with CF,

Submitted for publication.

we measured H_2 excretion in 14 CF patients before and after a standard dose of amylopectin.

Key words: Starch tolerance test, cystic fibrosis, breath hydrogen.

Patients and methods

Amylopectin* has been used instead of starch since the latter has to be boiled to become soluble and is difficult for children to consume. An amylopectin tolerance test (APT) was done in 14 children with CF, ages between 5 and 14 years. After an overnight fast unboiled amylopectin (1 g per kg) dissolved in water was administered. Palatability of the solution was corrected with a few drops of essence. Pancreatin administration at the day of investigation was withheld. H_2 estimations were done with 30 minutes intervals before and after administration of amylopectin, using our previously described method of measuring H_2 in mixed expired air (7) over a period of at least 2½ hours and in half of the children for at least 6 hours.

Additionally, tolerance tests with lactose, sucrose and protein hydrolysate (Purprotin**) were performed in these children. One or more of the latter tests were also done in 9 other CF patients.

Results

APT in the 14 children investigated was negative though 2 of them showed a moderate increase of 11 and 12 ppm H_2 respectively above base line values, indicating a subclinical degree of malabsorption (8).

An intriguing finding was that base line values were elevated on 26 out of 69 examination days (38%) in 15 out of 23 children with CF (65%), ranging from 11-70 ppm H_2, mean 24 ± 16 ppm.

In 14 out of 69 tests performed, a whimsical H_2 curve was found with uninterpretable fluctuations of H_2 excretion under or in the abnormal concentration range (> 20 ppm). This was clearly related to pre-existing elevated base line values in 13 out of 14 (93%) cases (Fig. 1). Hence, in 13 out of 15 patients (87%) with elevated fasting H_2 concentrations, respiratory H_2 excretion remained abnormal for several hours.

*Produced by Serva, Heidelberg, Germany.

**Produced by Nutricia, Holland.

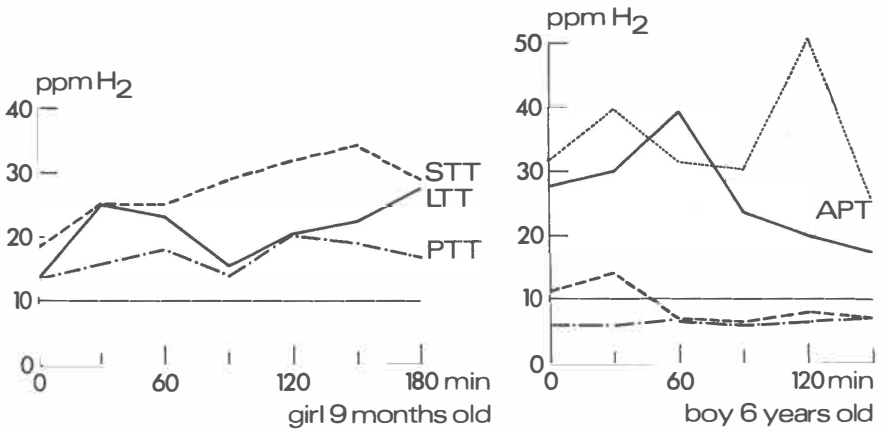

Fig. 1. Expired H_2 concentrations in mixed expiratory air samples in 2 children with cystic fibrosis, undergoing different tolerance tests. STT = sucrose tolerance test; LTT = lactose tolerance test; PTT = protein tolerance test; APT = amylopectin tolerance test. Horizontal bar delineates upper normal fasting H_2 concentration.

Discussion

These findings show that the H_2 breath test after challenge with amylopectin in children with CF does not contribute to the diagnosis of starch malabsorption since digestion and absorption of unboiled amylopectin in the present population did not give rise to a definite abnormal increase of H_2 in expired air.

In order to find out whether colonic microflora is able to generate H_2 from amylopectin, 0.5 g was added to a suspension of 3-4 g of freshly passed faeces in 0.9% NaCl, incubated at 37 °C, in rubber sealed 14 ml capacity glass tubes. After 2 hours a sample taken from the supernatant air in the test tubes, revealed the presence of large amounts of H_2 in contrast to the control tubes without added amylopectin. From these in vitro experiments, it may be expected that malabsorption of amylopectin should give rise to abnormal H_2 excretion.

Other methods of estimating starch malabsorption in CF patients also failed to demonstrate a significant malabsorption, even in the presence of steatorrhoea (4, 9). In 7 children with CF, repeated examinations revealed a slightly reduced absorption coefficient of starch (> 96%) as compared to normal children (> 99%). A low absorption coefficient of 90 and 82% respectively was found in 2 children and this correlated with very low

α-amylase activity of intestinal juice (4). Starch tolerance tests in adults with pancreatitis or pancreatic carcinoma, using blood glucose determinations, are more rewarding (11). The poor test results in children suffering from CF, using breath H_2 may be attributed to a sufficient residual amylase activity in most of them. It should also be realized that α-amylase is not exclusively produced by the pancreas but also by the salivary glands along the upper gastro-intestinal tract and that glucoamylase activity of the intestinal mucosa hydrolyses starch directly to glucose (3, 12).

Before the start of this study, we repeatedly noticed the presence of abnormal high fasting H_2 values in children with CF and in ulcerative colitis (UC) and Crohn's disease (CD). This contrasts with normal children who only rarely have fasting levels above 10 ppm H_2 in mixed expired air (10). Since these abnormal fasting levels tend to be maintained for several hours, interpretation of tolerance tests may lead to erroneous conclusions, especially since it cannot be excluded that an initial normal value may increase spontaneously during the test. Two patients with CF who had an abnormal H_2 excretion after lactose, had normal excretion at a repeat test. Since the APT in the 2 children with moderate H_2 increase was not repeated, we cannot be sure about the interpretation.

It is tempting to speculate about the origin of excess H_2 in CF and in inflammatory bowel disease. It has been shown previously that faecal lactic acid is increased in UC, which fact could not reasonably be explained by carbohydrate malabsorption (14). It may be a correct assumption that the increased production of mucus in UC and the finding of increased numbers of mucinase producing enterococci in UC, which are found to be 100-1000 times higher than in normals (13) leads to endogenous release of fermentation products such as lactic acid and H_2. It may therefore be speculated that similar events occur in the CF patient, due to the abnormal nature of his intestinal secretory products.

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References

1. Auricchio, S., Della Pietra, D. and Vegnente, A. Studies on intestinal digestion of starch in man. II. Intestinal hydrolysis of amylopectin in infants and children. Pediatrics, 39, 853 (1967).
2. Auricchio, S., Ciccimarra, F., Della Pietra, D. and Vegnente, A. Intestinal hydrolysis of starch. Mod. Probl. Pediat., 11, 22 (1968).
3. Auricchio, S. and Ciccimarra, F. Glucamylotic digestion of starch in human intestinal mucosa. Digestion and Intestinal Absorption, 4, 45 (1970).
4. Auricchio, S., Ciccimarra, F., De Vizia, B. Starch malabsorption. Proceedings International Congress of Pediatrics, Wien, 139 (1971).
5. Bond, J.H., Levitt, M.D.: Use of pulmonary hydrogen (H2) measurements to quantitate carbohydrate malabsorption. J. Clin. Invest., 51, 1219 (1972).
6. Dolmans, W. H_2-excretie in de uitademingslucht bij koolhydraatmalabsorptie. Thesis, Nijmegen, The Netherlands, 1978.
7. Douwes, A.C., Fernandes, J., Rietveld, W. Hydrogen breath test in infants and children: sampling and storing expired air. Clin. Chim. Acta, 82, 293 (1978).
8. Douwes, A.C., Fernandes, J. and Jongbloed, A.A. Diagnostic value of sucrose tolerance test in children evaluated by breath hydrogen measurement. Acta Paed. Scand., in press.
9. Fogel, M.R., Gray, G.M. Starch hydrolysis in man: an intraluminal process not requiring membrane digestion. J. Appl. Physiol., 35, 263 (1973).
10. Solomons, N.W. and Viteri, F., Rosenberg, L.H. Development of an interval sampling hydrogen (H_2) breath test for carbohydrate malabsorption in children: evidence for a circadian pattern of breath H_2 concentration. Ped. Res., 12, 816 (1978).
11. Sun, D.C.H. and Shay, H. An evaluation of the starch tolerance test in pancreatic insufficiency. Gastroenterology, 40, 379 (1961).
12. Thompson, D.L. Separation and characterization of human intestinal mucosal amylases. Gastroenterology, 48, 854 (1965).
13. Wiel-Korstanje, J.A.A. van der. Bifidobacterien en enterococcen in de darmflora van de mens. Thesis, Utrecht, The Netherlands (1973).
14. Weijers, H.A., Kamer, J.H. van de, Wauters, E.A.K., Pikaar, N.A. Diagnosis of malabsorption. Proceedings International Congress of Pediatrics, Wien (1971).

(A.C.D.) Children's Department, Academic Hospital of the Free University, De Boelelaan, Amsterdam, The Netherlands.

Chapter 8

SUGAR MALABSORPTION IN HEALTHY NEONATES ESTIMATED BY BREATH H_2

A.C. DOUWES, R.F. OOSTERKAMP, J. FERNANDES, T. LOS and A.A. JONGBLOED

From the Children's Department, Free University Hospital, Amsterdam, The Netherlands.

Summary

Carbohydrate malabsorption (CHM) in 110 healthy, fullterm neonates was studied by estimation of expired H_2 before and after a feed on the 6th or 7th day of life. CHM was assumed to be present if the infant excreted > 20 parts per million (ppm) H_2. The frequency of CHM in breastfed infants was 25% (12 out of 49), in infants fed on a 7.5% lactose formula 31% (11 out of 35), in infants on a formula containing 1% lactose and 7.3% maltodextrin 15% (4 out of 26). Statistically these differences in frequency were not significant.

Peak H_2 concentration of the malabsorbers in each group, indicating the degree of CHM, was 64, 52 and 32 ppm respectively. The degree of CHM did not differ significantly between the breastfed and the high lactose formula group, but both groups differed from the low lactose group ($p < 0.01$).

H_2 excretion was studied for 5 months in an exclusively breastfed infant. In the first 2 months high concentrations were found and the infant produced 3-5 stools per day. In the next 3 months, however, most H_2 estimations were normal and only 1-2 stools per week were passed. With the introduction of solids, daily bowel movements promptly reocurred.

Frequency of CHM in newborns is rather high and is primarily related to the lactose intake. In contrast to previous reports, frequency and degree of CHM were found to be of the same order in breastfed infants and in infants fed on a high lactose formula.

Archives of Disease in Childhood, in press.

The occurrence of carbohydrate malabsorption (CHM) in normal, term-born neonates on breast milk or on cow's milk based formulas, may be assumed from the finding of more than 0.5% reducing substances in the faeces (Davidson and Mullinger, 1970; Counahan and Walker-Smith, 1976; Whyte et al., 1978) or from the finding of more than 15 mg% reducing substances in the urine (Haworth and McCredie, 1956). The mean "normal" excretion of lactose in the urine of 88 neonates between the 3rd and 7th day of life ranges between 20-50 mg% (Bickel, 1961).

Sugar chromatography on stools with excess reducing substances usually reveals the presence of lactose, glucose and galactose (Counahan & Walker-Smith, 1976; Whyte et al., 1978). This pattern is consistent with incomplete lactose absorption since non-absorbed lactose is split by the faecal flora into its component monosaccharides (Lindquist and Wranne, 1976).

Lactose malabsorption has been reported to be more frequent in breastfed than in bottlefed infants, even when lactose concentrations of the milks are similar (Davidson and Mullinger, 1970; Counahan and Walker-Smith, 1976; Whyte et al., 1978). These findings may have practical implications from the nutritional point of view as well as in establishing a non-pathogenic type of large bowel microflora (Bullen et al., 1976; Heine et al., 1977). However, the reported differences of CHM in bottle- and breastfed infants may have been affected by differences in bacterial fermentation of the non-absorbed sugars and by contamination of the stools with lactose-containing urine.

It has recently been found that malabsorption of lactose and other sugars can easily be detected by estimation of H_2 in expired air in adults (Calloway et al., 1969; Levitt, 1969) and also in children (Maffei et al., 1977; Fernandes et al., 1978; Perman et al., 1978; Douwes et al., 1979). Using a procedure for interval sampling of mixed expired air adapted for infants (Douwes et al., 1978), we have studied the frequency and the degree of CHM in 110 newborns, fed on one of three feeding regimes. The main objective of this study was to find out whether the reported difference of CHM between bottlefed and breastfed infants could be confirmed.

Patients and methods

We examined 110 healthy full-term infants in the maternity ward of our hospital. Informed consent was obtained from the parents of all

Table 1. Constituents of the milks used in this study in gram per 100 ml. Fats in the formula feedings are derived from corn oil.

	breast milk	Almiron M2-B	Almiron A-B
lactose	6.6-7.1	7.5	1
maltodextrin	–	–	7.3
fat	2.9-3.5	3.5	2.9
protein	1.1-1.4	1.5	1.8

children studied. The infants were fed exclusively one of three milks: breast milk (n = 49), cow's milk based formula containing 7.5% lactose (Almiron M2-B*) (n = 35) or 1% lactose and 7.3% maltodextrin (Almiron A-B*) (n = 26). The composition of the formulas is recorded in Table 1. Infants on mixed feedings were not studied but breastfed infants whose feeding had been supplemented with glucose solution during the first few days after birth have been included. Mothers who breastfed their babies were instructed to avoid fruit and fruit juices on the day before and during the examination, since fruit sugars may be excreted into the breast milk.

To avoid the influence of large differences in intake of milk per kg body weight, actual body weight and ingested volume of the formula feedings was recorded. In the breastfed group ingested volume was estimated by test weighing of the infant before and after the feed. Serial H_2 estimations were done on the 6th or 7th day of life, starting at 9 a.m., three hours after the first feeding of that day. The second breath sample was taken at 10 a.m., just before the second feeding, the third and fourth at 11 a.m. and at noon respectively. Duplicate breath samples were stored in vacutainers for gaschromatographical analysis. The details of the procedure have been presented previously (Douwes et al., 1978). In children with lactose intolerance, an increase above base-line value of $>$ 10 ppm H_2 correlates positively with the occurrence of clinical symptoms (Barr et al., 1978, Douwes et al., 1978). Since the frequent feedings in the study group did not allow to measure H_2 excretion during fasting, which is below 10 ppm in older children (Barr, 1979; personal experience), a total concentration of $>$ 20 ppm H_2 in mixed expired air has been assumed to be a safe indication for the presence of CHM in these neonates.

*Manufactured by Nutricia, Zoetermeer, The Netherlands.

Results

All 27 infants with CHM were caucasian, 7 of them (26%) weighed more than 3500 g, 13 (48%) weighed more than 3000 g and 7 (26%) weighed more than 2500 g, the lowest weight being 2575 g. Diarrhoea did not occur, though all infants passed two or more loose stools per day. The mean volume of milk ingested (ml/kg body weight) was not different between the three feeding groups and only small individual differences were found.

The majority of the infants with CHM had elevated H_2 concentration in the first breath sample. This was usually followed by a further increase, though some remained on a more or less constant level and 2 infants had decreasing concentrations towards the end of the sampling period. Peak H_2 excretion of each infant is recorded in Fig. 1. The frequency of CHM in the breastfed group was 25% (12/49), in the 7.5% lactose formula group 31% (11/35), in the 1% lactose formula group with 7.3% maltodextrin 15% (4/26). These frequencies are not statistically different. The relative degree of CHM, measured as peak H_2 excretion, is not different for breastfed infants and those on the 7.5% lactose formula. But both of these groups have a significant higher degree of CHM as compared to the group on the 1% lactose and 7.3% maltodextrin formula ($p < 0.01$, Table 2).

H_2 excretion in the breastfed infant during the first 150 days of life changed through the day (Fig. 2) and varied markedly on different days (Fig. 3). The highest concentrations, however, were found in the first two months. Stool frequency in the first 2 months was 3-5 per day. In the next 3 months, the baby still being exclusively breastfed, H_2 excretion became normal or moderately elevated (Fig. 3). Only 1-2 stools per week were passed in the last 3 months and no signs of constipation occurred. Introduction of solids at the age of 6 months immediately resulted in daily passage of 1-2 well-formed stools.

Discussion

The present study confirms earlier observations, based on the finding of excess reducing substances in the stools ($> 0.5\%$) or in the urine (> 15 mg%), that a considerable number of neonates have some degree of CHM (Haworth and McCredie, 1956; Davidson and Mullinger, 1970; Counahan and Walker-Smith, 1976; Whyte et al., 1978). The amount of

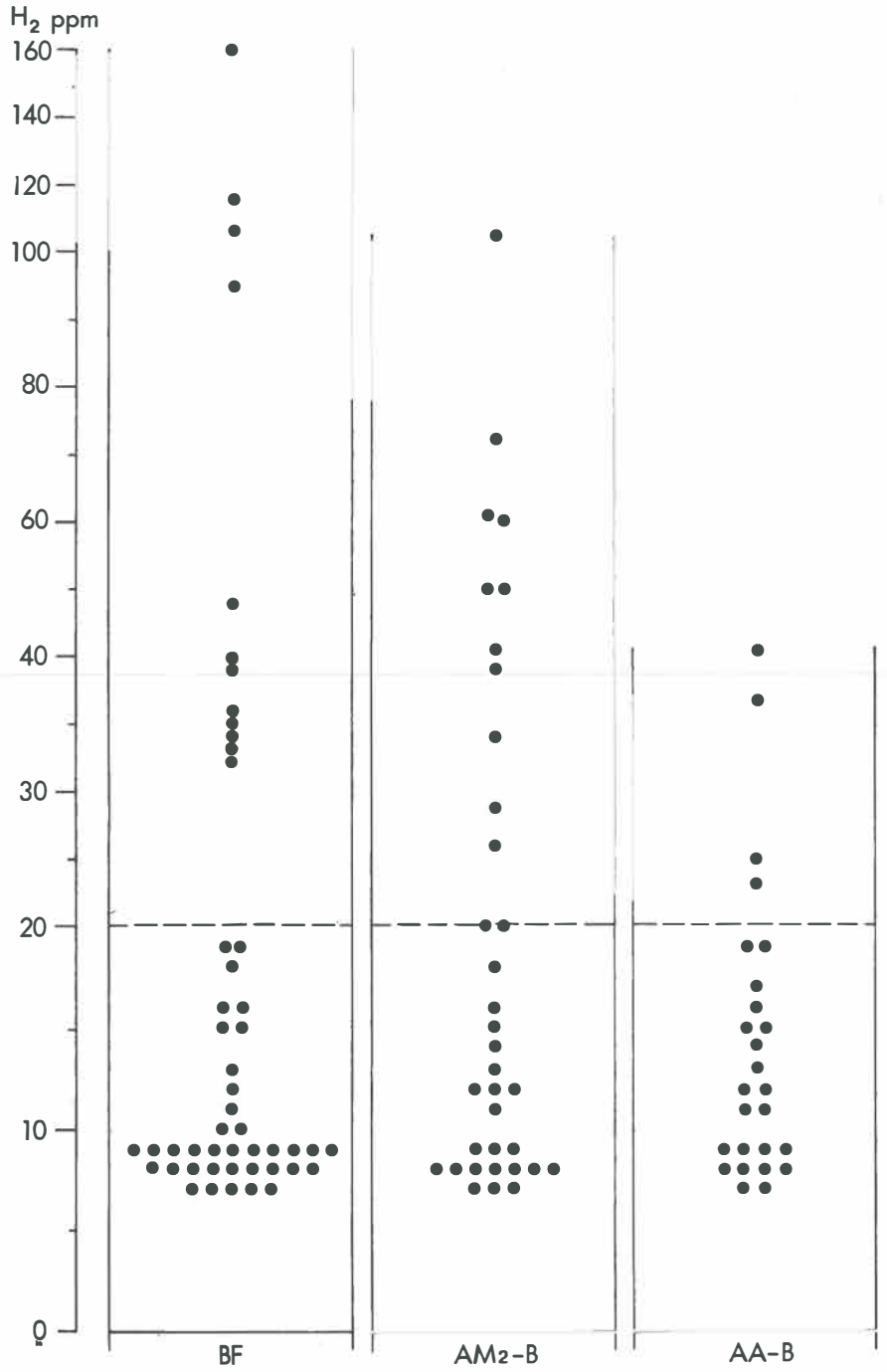

Fig. 1. Peak H_2 excretion in 110 normal, 6- or 7-day-old infants on one of three feeding regimes. Horizontal bar delineates upper normal value. BF: breastfed group; AM2-B: 7.5% lactose formula group; AA-B: 1% lactose with 7.3% maltodextrin formula group.

Table 2. Frequency and relative degree of carbohydrate malabsorption in three groups of neonates on different feeding regimes.
ns: not significant different.
s: significant different from breastfed and AM2-B group ($p < 0.01$).

	breast milk	Almiron M2-B	Almiron A-B
frequency:			
number of infants	12/49	11/35	4/26
percentage	24.5	31.4 (ns)	15.4 (ns)
95% confidence limits	15-39	18-49	7-34
mean ppm H_2 malabsorbers	64	52 (ns)	32 (s)
SD	43	23	9

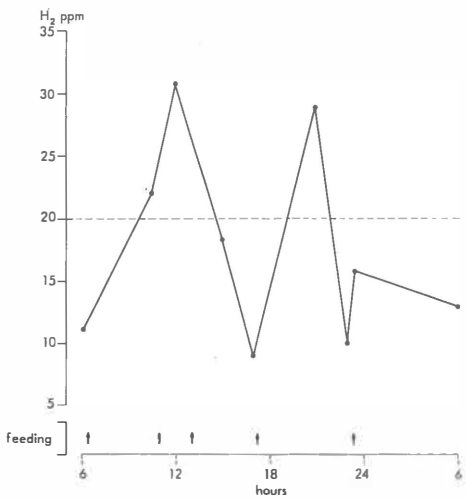

Fig. 2. Variation in H_2 excretion over a 24 hr period in an exclusively breastfed infant, age 28 days. Feedings indicated by arrows. Horizontal bar delineates upper normal value for adults and older children.

sugars found in excreta gives some indication about the degree of CHM which tends to be higher in breastfed infants than in bottlefed infants (Counahan and Walker-Smith, 1976; Whyte et al., 1978). Firm conclusions regarding different degrees of CHM between these groups however have not been made.

It has been shown previously that H_2 production is primarily related to the amount of fermentable substrate in the colon (Levitt, 1969) and

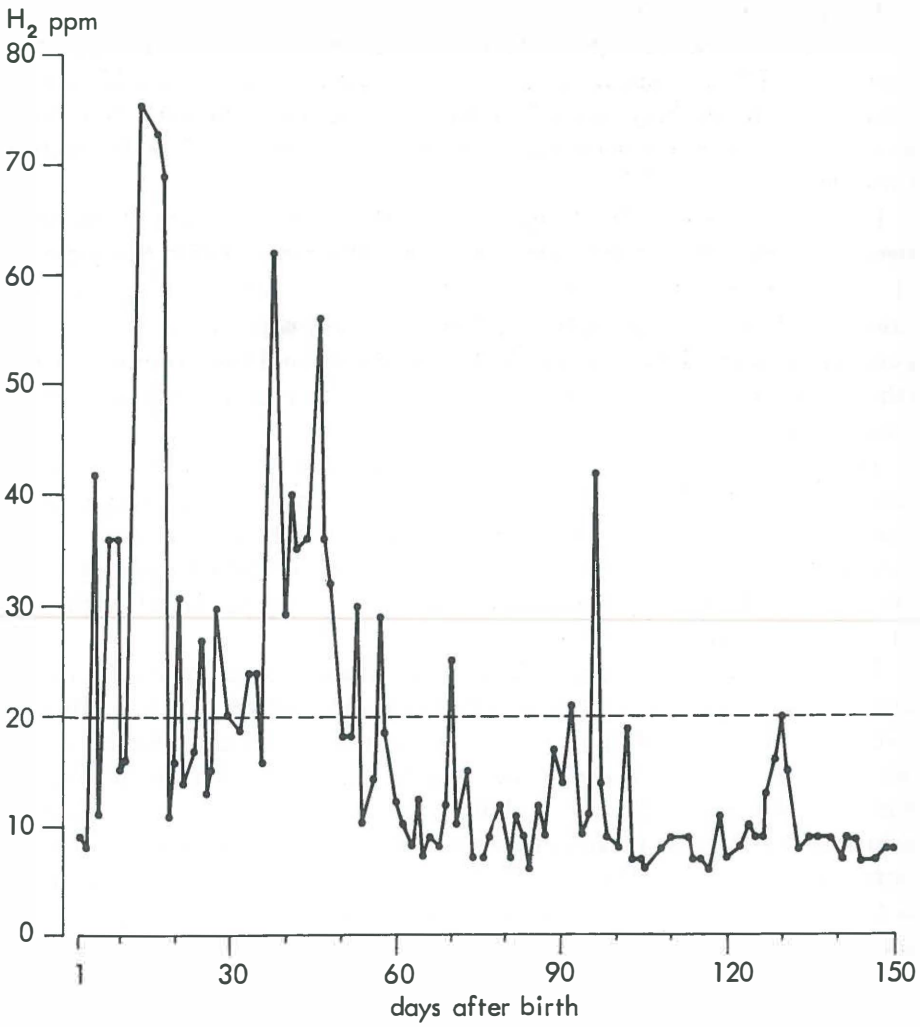

Fig. 3. H_2 excretion over 5 months in an exclusively breastfed infant. Horizontal bar delineates upper normal value, for adults and older children.

this amount can be calculated from the expired H_2 (Bond and Levitt, 1972). On the other hand, H_2 response to the same dose of lactose may differ considerably between individual lactose malabsorbers (Payne-Bose et al., 1977). Yet, mean H_2 response of a group of lactose malabsorbers to different lactose doses differs significantly ($P < 0.002$, Payne-Bose et al., 1977). It therefore seems justified to use mean H_2 concentrations of

different feeding groups to indicate dietary influence on the degree of CHM. In contrast to reports based on estimation of faecal sugar, the degree of CHM as indicated by mean H_2 response, was not different in breastfed infants from those on the 7.5% lactose formula, but both groups were different from those on the 1% lactose, 7.3% maltodextrin formula ($p < 0.01$).

Reports concerning the frequency of CHM in neonates are conflicting, figures ranging from 0-50% (Haworth and McCredie, 1956; Soeparto et al., 1972). The true frequency of CHM in neonates is difficult to establish since CHM may be present intermittently as judged from the lactose content in urine (Haworth and McCredie, 1956) and the excretion of H_2 (this study (Fig. 2, 3). It should be noted that our present data are merely cross-sectional (Fig. 1).

The real frequency might therefore be considerably higher than these studies suggest. This is supported by the findings of our longitudinal study (Fig. 3). A relation may exist between CHM and frequency of defaecation as suggested by the marked decrease of stool frequency, synchronous with the decreasing concentrations of expired H_2 after the age of 2 months (Fig. 3).

Lactose is commonly implicated as the major source of excess faecal and urinary sugar in newborns (Haworth and McCredie, 1956; Bickel, 1961; Davidson and Mullinger, 1970; Counahan and Walker-Smith, 1976; Whyte et al., 1978), though other sugars have also been found (Counahan and Walker-Smith, 1976; Whyte et al., 1978). The accumulating evidence for lactose malabsorption in a considerable proportion of newborns seems to contradict the finding that brush-border lactase activity is fully mature at the time of birth though its maximum is reached only shortly before delivery (Grand et al., 1976).

Presumably the total splitting capacity of the neonatal small bowel is reduced due to a smaller surface area, as compared to older infants. Previous studies, based on estimation of faecal sugar, however, emphasise the influence of the nature of the feeding, suggesting that frequency and degree of CHM in breast-fed infants is higher than in formula-fed neonates, even when these formulas contain lactose in amounts comparable to human milk (Davidson, 1970; Counahan and Walker-Smith, 1976; Heine et al., 1977; Whyte et al., 1978). Our study fails to confirm this interpretation since frequency of abnormal H_2 excretion in breastfed infants did not differ significantly from that in bottlefed infants and was even lower in the breastfed group than in the high lactose formula group (Table 2).

Our findings, based on breath H_2 measurements, indicate that CHM in healthy, fullterm neonates is primarily due to an age-related maturation process of lactose digestion and absorption, rather than to the nature of the feeding.

Acknowledgements

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References

Barr, R.G., Becker, M.C., Heymann, P.W. and Watkins, J.B. Lactose malabsorption, abdominal pain and lactose ingestion in a multiethnic school population. Gastroenterology, 74, 1006 (1978).

Barr, R.G. Personal communication. (1979).

Bickel, H. Mellituria, a paper chromatographic study. Journal of Pediatrics, 59, 641-656 (1961).

Bond, J.H., Levitt, M.D. Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate malabsorption. Journal of Clinical Investigation, 51, 1219-1225 (1972).

Bullen, C.L., Tearle, P.V. and Willis, A.T. Bifidobacteria in the intestinal tract of infants: an in-vivo study. Journal of Medical Microbiology, 9, 325-333 (1976).

Calloway, D.H., Murphy, E.L. and Bauer, D. Determination of lactose intolerance by breath analysis. American Journal of Digestive Diseases, New Series, 14, 811-815 (1969).

Counahan, R. and Walker-Smith, J. Stool and urinary sugars in normal neonates. Archives of Disease in Childhood, 51, 517-520 (1975).

Davidson, A.G.F. and Mullinger, M. Reducing substances in neonatal stools detected by Clinitest. Pediatrics 46, 632-635 (1970).

Douwes, A.C., Fernandes, J. and Rietveld, W. Hydrogen breath test in infants and children: sampling and storing expired air. Clinica Chimica Acta, 82, 293-296 (1978).

Douwes, A.C., Fernandes, J., Degenhart, H.J. Improved accuracy of lactose tolerance test in children, using expired H_2 measurement. Archives of Disease in Childhood, 53, 939-942 (1978).

Douwes, A.C., Fernandes, J. and Jongbloed, A.A. Diagnostic value of sucrose tolerance test in children, evaluated by breath hydrogen measurement. Acta Paediatrica Scandinavica (in press).

Fernandes, J., Vos, C.E., Douwes, A.C., Slotema, E. and Degenhart, H.J. Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. American Journal of Clinical Nutrition, 31, 597-602 (1978).

Grand, R.J., Watkins, J.B. and Torti, F.M. Development of the human gastrointestinal tract: a review. Gastroenterology 70, 790-810 (1976).

Haworth, J.C. and McCredie, D. Chromatographic separation of reducing sugars in the urines of newborn babies. Archives of Disease in Childhood, 31 189-190 (1956).

Heine, W., Zunft, H.J., Möller-Bethow, W. and Grütte, F.K. Lactose and protein absorption from breast milk and cow's milk preparations and its influence on the intestinal flora. Acta Paediatriaca Scandinavica, 66, 699-703 (1977)

Levitt, M.D. Production and excretion of hydrogen gas in man. New England Journal of Medicine, 281, 122-127.

Lindquist, B.L. and Wranne, L. Problems in analysis of faecal sugar. Archives of Disease in Childhood, 51, 319-321 (1976).

Maffei, H.V.L., Metz, G. Bampoe, S.V., Shiner, M. and Brook, C.G.D. Lactose intolerance detected by the hydrogen breath test in infants and in children with chronic diarrhoea. Archives of Disease in Childhood, 52, 766-771 (1977).

Payne-Bose, D., Welsh, J.D. Gearhart, H.L. and Morrison, R.D. Milk and lactose-hydrolyzed milk. American Journal of Clinical Nutrition, 30, 1799-1810 (1977).

Perman, J.A., Barr, R.G., Watkins, J.B. Sucrose malabsorption: diagnosis by interval breath hydrogen. Journal of Pediatrics, 93, 17-22 (1978).

Whyte, R.K., Homer, R. and Pennock, C.A. Faecal excretion of oligosaccharides and other carbohydrates in normal neonates. Archives of Disease in Childhood, 53, 914-915 (1978).

Chapter 9

SUMMARY AND DISCUSSION

The work of Doris Calloway and Michael Levitt has been the start for the development of a non-invasive, non-isotope and highly sensitive instrument in the study of carbohydrate digestion and absorption in man. Several paediatricians soon realized its potential usefulness in paediatrics and stimulated its application in children.

This study shows that the rebreathing procedure can be used in older children for the detection of lactose intolerance due to lactase deficiency, demonstrating at the same time that a low lactose intake in these patients avoids the manifestation of clinical symptoms notwithstanding the fact that H_2 excretion still indicates the presence of malabsorption (chapter 2).

The development of an interval sampling method using small amounts of mixed expired air, widened the application of the breath H_2 test to children of all ages without affecting its discriminatory value (chapter 3).

That the conventional sugar tolerance tests with estimation of blood glucose rise often lead to erroneous conclusions, has been suspected by many clinicians. Simultaneous measurement of blood glucose and expired hydrogen confirms that this suspicion is correct, especially with the lactose tolerance test. Estimation of blood sugar is of little value in discriminating between lactose tolerance and intolerance and of no value at all for the detection of lactose malabsorption, i.e. a low degree of lactose loss into the large bowel without or with minor clinical symptoms. Hence, use of the H_2 breath test will help to avoid unnecessary dietary manipulation, indicating only those children who may benefit from lactose restriction (chapter 4).

Many children with recurrent diarrhoea, with or without abdominal pain, are urged to consume diets free of lactose as well as from sucrose. Though elimination of lactose is beneficial in some of them, this is not the case for sucrose, as has been shown in chapter 5. Therefore, the commonly prescribed lactose-sucrose-free diets can be simplified to lactose-free ones only. The rare condition of isolated sucrase-isomaltase deficiency can be diagnosed easily with the breath H_2 test, followed by appropriate dietary treatment (chapter 5).

The extremely rare occurrence of hereditary glucose-galactose malabsorption provided a unique opportunity to study H_2 excretion as a result of malabsorption of monosaccharides. Administration of dif-

ferent amounts of the same sugar in this infant also demonstrated the dose-related H_2 response (chapter 6).

Application of the breath H_2 test for the detection of malabsorption of polysaccharides (amylopectin) in children with cytic fibrosis, however, was not rewarding. Yet, the finding of abnormal high fasting H_2 levels in most of these patients is intriguing and awaits further elucidation (chapter 7).

New questions are also brought up by the results of measuring H_2 excretion in healthy neonates after consumption of whole milks (chapter 8). The expected difference in frequency and degree of carbohydrate malabsorption between breastfed babies and those fed a high lactose cow's milk formula was not encountered. These findings contrast with previous studies based on faecal excretion of sugars, indicating a higher frequency and degree of malabsorption in breastfed infants as compared to bottlefed babies. The presence of lactose in the infantile large bowel undoubtedly is a supply of energy to the rapidly expanding microflora but does not seem to occur in breastfed infants only.

Apart from this teleogical speculation concerning the usefulness of some degree of carbohydrate malabsorption in the first few weeks of life, the mechanism giving rise to it, remains unclear, since it has been shown that disaccharidase activities in the neonatal small bowel mucosa measured per unit of surface, are fully mature at the time of birth.

The present work is only an intermediate step in the rapidly expanding experience concerning the many diagnostic and research facilities provided by the breath H_2 test.

SAMENVATTING EN DISCUSSIE

De onderzoekingen van Doris Calloway en Michael Levitt vormen het begin van de ontwikkeling van een niet invasieve, geen stralenbelasting veroorzakende en zeer gevoelige methode voor het onderzoek van de vertering en resorptie van koolhydraten bij de mens. Verschillende kinderartsen beseften spoedig de mogelijkheden ervan voor de kindergeneeskunde en stimuleerden de toepassing bij kinderen.

Aangetoond wordt dat de rebreathing methode bij oudere kinderen kan worden toegepast voor de diagnose van lactose-intolerantie ten gevolge van lactase-deficientie. Tevens wordt aangetoond dat lactose-intolerante kinderen kleine hoeveelheden lactose in de vorm van melk kunnen verdragen zonder klinische symptomen terwijl de uitscheiding van waterstof ook dan nog duidt op de aanwezigheid van malabsorptie (hoofdstuk 2).

De ontwikkeling van een methode waarbij gebruik wordt gemaakt van kleine hoeveelheden gemengde uitademingslucht, maakt toepassing van de waterstof-ademproef mogelijk bij kinderen van alle leeftijden (hoofdstuk 3).

Dat de gebruikelijke suiker-belastingsproeven met bepaling van de stijging van het glucosegehalte van het bloed veelal tot verkeerde konklusies aanleiding geven werd door vele clinici reeds vermoed. Gelijktijdige bepaling van bloedglucose en uitgeademde waterstof bevestigt de juistheid van dit vermoeden, met name ten aanzien van de lactose tolerantieproef. Bepaling van bloedglucose heeft nauwelijks waarde voor het onderscheid tussen lactose-intolerantie en normale resorptie van lactose, en in het geheel geen waarde voor het aantonen van lactose-malabsorptie. Onder lactose-malabsorptie wordt in dit verband verstaan een gering tot matig verlies van lactose in de dikke darm zonder dat klinische verschijnselen optreden.

Toepassing van de waterstof-ademproef in de kindergeneeskunde voorkomt onnodige dieetvoorschriften, aangezien alleen die kinderen worden geselecteerd die baat kunnen hebben bij vermindering van het lactosegehalte in hun voeding (hoofdstuk 4).

Veel kinderen met intermitterende diarree, al dan niet gepaard gaand met buikpijnklachten, krijgen een lactose- en saccharose-arme voeding voorgeschreven. Hoewel vermindering van het lactosegehalte voor een aantal van deze kinderen zinvol is, blijkt dit niet te gelden voor saccharose, zoals wordt aangetoond in hoofdstuk 5. De veel gebruikte combi-

natie van lactose-saccharose-arme voeding kan daarom worden vervangen door alleen lactose-arme, hetgeen de naleving van het dietvoorschrift vereenvoudigt.

De zeldzame primaire sucrase-isomaltase deficientie kan gemakkelijk worden aangetoond met de waterstof-ademproef gevolgd door een aangepaste dieetbehandeling.

Een geval van de zeer zeldzame aangeboren glucose-galactose malabsorptie bood de unieke gelegenheid om de waterstofuitscheiding te bestuderen als gevolg van malabsorptie van enkelvoudige suikers. Ook bij deze patient werd aangetoond dat er een relatie bestaat tussen de concentratie van de uitgeademde waterstof en de toegediende hoeveelheid suiker (hoofdstuk 6).

Toepassing van de waterstof-ademproef voor het opsporen van malabsorptie van meervoudige suikers (amylopectine) bij kinderen met pancreasfibróse bleek niet lonend. Niettemin is de bevinding dat de meeste (Garson et al., Circulation 59: 1232, 1979).
van waterstof hebben, intrigerend en vraagt om opheldering van de oorzaak (hoofdstuk 7).

Nieuwe vragen worden ook opgeroepen door de resultaten van het onderzoek naar de waterstof-uitscheiding door gezonde pasgeborenen na een voeding (hoofdstuk 8). Het verwachte verschil in voorkomen en mate van suiker-malabsorptie tussen kinderen met borstvoeding en kinderen met flesvoeding met hoog lactosegehalte, werd niet gevonden. Deze uitkomsten zijn in tegenspraak met de resultaten van eerdere onderzoekingen, gebaseerd op de uitscheiding van suiker in faeces die suggereren dat koolhydraat-malabsorptie bij borstvoeding vaker zou voorkomen dan bij flesvoeding. Ongetwijfeld betekent de aanwezigheid van lactose in de dikke darm een energiebron voor de zich snel ontwikkelende darmflora, maar dit is niet beperkt tot kinderen die borstvoeding ontvangen. Afgezien van deze speculatie over de teleologische betekenis van enige koolhydraatmalabsorptie gedurende de eerste levensweken, blijft de oorzaak ervan onduidelijk, aangezien de enzymaktiviteit van de verschillende disaccharidasen in het slijmvlies van de dunne darm reeds maximaal is bij de geboorte.

Het huidige onderzoek is een kleine bijdrage aan de snel toenemende ervaring met de vele diagnostische en onderzoeksmogelijkheden die de waterstof-ademproef te bieden heeft.

CURRICULUM VITAE

De samensteller van dit proefschrift werd op 12 februari 1940 te Utrecht geboren. In 1957 behaalde hij het MULO-diploma te Ermelo, in 1959 het HBS-B diploma te Harderwijk. Na een onderbreking voor het vervullen van de miliatire dienstplicht studeerde hij geneeskunde aan de Vrije Universiteit te Amsterdam. In 1967 werd het kandidaatsexamen afgelegd, in 1969 het doctoraal examen. Hij was van 1967 tot 1969 als student-assistent verbonden aan het Laboratorium voor Anatomie en Biomechanica (Hoofd: Prof. Dr. F. van Faassen). Na het artsexamen in 1971 specialiseerde hij zich tot kinderarts op de kinderafdeling van de Vrije Universiteit (Hoofd: Prof. Dr. T.D. Stahlie) en werd op 1 juni 1975 alszodanig ingeschreven in het specialistenregister.

De Vakgroep Kindergeneeskunde van de Vrije Universiteit stelde hem in de gelegenheid om zich verder te bekwamen in de kindergastroënterologie. Dit gebeurde vanaf januari 1975 tot september 1977 in het Sophia Kinderziekenhuis en Zuigelingenkliniek van de Erasmus Universiteit te Rotterdam (Hoofd: Prof. Dr. H.K.A. Visser) onder leiding van Dr. J. Fernandes, nu hoogleraar kindergeneeskunde te Groningen. In deze periode vond het grootste deel van het in dit proefschrift beschreven onderzoek plaats. Sinds 1 juni 1978 is hij als wetenschappelijk hoofdmedewerker in volledig dienstverband werkzaam op de kinderafdeling van het Academisch Ziekenhuis der Vrije Universiteit met als voornaamste taak de kindergastroënterologie. Tevens is hij gastroënterologisch consulent voor de geaffilieerde kinderafdeling van het Onze Lieve Vrouwe Gasthuis te Amsterdam (Hoofd: Dr. L.H.B.M. van Benthem).

Hij is getrouwd met Dineke Ozinga en heeft drie kinderen: Martine, Hilde en Sara.